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THE SECRETION OF SODIUM AND UPTAKE OF POTASSIUM BY ISOLATED UTERINE SEGMENTS MADE SODIUM-RICH

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Studies of the electrolyte composition and ionic exchanges of uterine wall and muscle (Daniel & Daniel, 1957, 1959; Daniel, 1958) provided evidence that the chloride distribution across the cell membranes might be passive, but that the accumulation of potassium and extrusion of sodium could not be so explained if the membrane potentials of uterine cells had been accurately measured (Daniel & Singh, 1958). Evidence was also presented that a substantial fraction of the sodium and probably also of the potassium was bound (associated with intracellular anions so as not to be readily diffusible) and unable to contribute to the osmotic activity of tissue water.

The first purpose of the present study was to obtain evidence from net movements of sodium and potassium which, together with knowledge of membrane potentials, would provide proof of the occurrence of active transport in the uterus. When this proved possible, studies of the effects of variations in the ionic contents of the external fluid were carried out to analyse the mechanism for active transport. Finally, the nature of the dependence of active transport in the uterus on metabolism was investigated. These last investigations are dealt with in the following article (Daniel & Robinson, 1960).

Uterine segments from non-pregnant rabbits furnished the material for most of these experiments. Parallel experiments were run in most instances with uterine tissue from pregnant cats. These two types of uterine tissue were selected because they possess extreme differences in electrical properties (Daniel, 1960). In the non-pregnant rabbit uterus action potentials are conducted only rather slowly and over very short distances. Occurrence of action potentials is not regularly related to over-all tension increments. In contrast, in the pregnant cat uterus action potentials spread regularly over distances of several centimetres, always occurring before and during rapid increase in tension and disappearing before relaxation. The rabbit uterine tissues were of two types; those from animals pre-treated with oestrogen alone and those from animals pre-treated with both oestrogen and progesterone.

METHODS

Preparation of animals and removal of tissues. Immature female albino rabbits of the New Zealand strain weighing less than 3 lb. (1·4 kg) were treated with oestrogen (diethylstilboestrol, 125 μg/day, subcutaneously) for 6 days or for 9 days. In the latter instances progesterone (2 mg/day, subcutaneously) was administered for the last 3 days. Rabbits were killed by a blow over the foramen magnum, their abdomens rapidly opened and the uteri excised.

Pregnant cats were obtained under circumstances that necessitated their survival. They were therefore anaesthetized with pentobarbital sodium, and the uterus removed as rapidly as possible after ligatures had been placed around its blood vessels.

Experimental procedure. Rabbit uteri were cut into several segments and rapidly weighed. An average segment weighed 0·2 g and was 1·5 cm long and 0·5 cm in diameter. One segment was taken for analysis as a fresh control. For identification purposes threads with varying numbers of knots were then tied around the remaining segments, which were suspended in 25 ml. or 35 ml. K-free Krebs–Ringer solution and placed in the cold at 4° C during the night without aeration. The next day all the segments were reweighed and one segment was removed for analysis of the extent of ion exchanges in the cold. The medium to which the uterine pieces had been exposed in the cold was also analysed.

The uterine segments then were returned for 120 min into experimental media of differing composition, which were warmed to 37° C and aerated with 95 % O_2 + 5 % CO_2. The segments were then weighed a final time and placed in the oven to be dried and subsequently analysed.

Uteri from pregnant cats were first opened and the foetuses and placentae were removed and the uterine walls then cut into strips. These strips were then treated like the segments of rabbit uterus.

Media. The compositions of the principal types of media used are shown in Table 1. The specific gravities, and the concentrations of water and solids in the media used when the concentration of Na was varied, are shown in Table 2 as measured at 24° C. The specific gravities and the grams of solids and water per litre of these solutions are given, because of their importance in evaluating the changes in tissue weight observed in these solutions. Specific gravities were determined in triplicate. Additional variations were made in the constituents of the principal media, but these will be discussed in relation to the experiments in which these media were used.

Analysis of tissues. The analytical methods have been described in detail elsewhere (Daniel & Daniel, 1957; Daniel, Dodd & Hunt, 1959). Briefly, water concentrations in uterine tissues were determined by drying for 5 days or more at 100° C and reweighing the tissues. The tissues were then ground, reweighed, and divided into portions for duplicate chloride analysis and analysis of sodium and potassium. Tissue chloride was analysed by a modification of the micro-electrometric method described by Whittam (1955). Chloride in the media was measured by the method of Asper, Shales & Shales (1947). Sodium and potassium were measured with a Janke flame spectrophotometer, with lithium as an internal standard.

Calculation and expression of results. Tissue electrolyte concentrations were expressed in terms of the final wet weights. In addition, the sum of sodium and potassium per litre of tissue water $[Na+K]_t/[H_2O]_t$ was calculated as an indication of the movements of cations relative to water.

Changes in the solids and water concentrations relative to the fresh weights were estimated in the following way. The weights of solids and of water per kilogram in the experimental pieces were calculated, assuming them to be identical initially to those in the fresh control. The changes in solids and water were then determined by comparison of their values at the end of the experiment to those calculated for the original conditions. The changes were then expressed relative to the original fresh weight (f.wt).

The S.E.s of the means have been calculated for all analytical and all derived values. They

are not given in the tables because of the large number of values to be presented and because most of the changes were striking. Significant differences ($P < 0.05$) will be so designated in the text. Over three thousand tissues have been analysed and the results under similar experimental conditions have been remarkably consistent. Statistical evaluation of the analytical values for fresh tissues, and for tissues exposed during the night to cold, revealed very small variances between experiments carried out at different times. The results obtained at various times have been grouped and compared with the cumulated average values for both the fresh and the K-depleted controls from all the individual experiments. The loss of potassium and gain of sodium, chloride and water in the cold was relatively uniform, despite

TABLE 1. Composition of media (mM)

	Krebs–Ringer solution	K-free Krebs's solution	PO_4-free Krebs's solution	Saline*	Saline* bicarbonate	Saline $-KHCO_3$	Choline Cl– $KHCO_3$	Sucrose $-KHCO_3$
Sodium	138·55	143·17	137·39	158·17	158·17	158·0	—	—
Chloride	125·05	125·04	125·05	158·17	136·26	158·0	158·0	—
Choline	—	—	—	—	—	—	158·0	—
Sucrose	—	—	—	—	—	—	—	287·03†
Potassium	4·63	—	4·63	—	—	5·0	5·0	5·0
Phosphate	1·16	1·16	—	—	—	—	—	—
Bicarbonate	21·91	21·91	21·91	—	21·91	5·0	5·0	5·0
Calcium	2·47	2·47	2·47	—	—	—	—	—
Magnesium	1·16	1·16	1·16	—	—	—	—	—
Sulphate	1·16	1·16	1·16	—	—	—	—	—
Glucose	49·20	49·20	49·20	—	49·20	49·20	49·20	49·20

* In some experiments sufficient potassium (as KCl) was added to these solutions to make final potassium concentrations of either 5 or 25 mM (see text).

† Approximate osmotic equivalent of 158 mM-NaCl (Conway & McCormach, 1953).

TABLE 2. Specific gravities and concentrations of water and solids

[Na] (mM)	Choline medium			Sucrose medium		
	(g/l.)	(g solids/l.)	(g H_2O/l.)	(g/l.)	(g solids/l.)	(g H_2O/l.)
0	1001·75	31·41	970·34	1036·06	107·50	928·56
20	1001·66	29·79	971·87	1032·17	96·25	935·92
40	1002·66	28·17	974·49	1027·98	85·00	942·98
80	1003·66	24·93	978·73	1020·69	62·50	958·19
100	1003·95	23·30	980·65	1016·40	51·25	965·15
158	1006·32	18·60	987·72	1006·32	18·60	987·72

marked differences in the concentrations of leached potassium which accumulated in the medium during the night. The average concentration of potassium in the leaching medium in eighty-four experiments on all types of rabbit uteri was 3·32 m-equiv/l. $\pm$ 0·15 (1·72–7·43) and in twelve experiments on uteri from pregnant cats it was 6·84 m-equiv/l. $\pm$ 0·68 (2·73–10·73). Apparently the decreased temperature and consequent depression of metabolism rather than the extent of diminution of external potassium concentration determined the extent to which ion exchanges took place. Subsequent experiments suggested that the lack of aeration could have had little effect (Daniel & Robinson, 1960). Tissues from oestrogen and from oestrogen-and-progesterone-treated groups have been combined in most instances. This was done to facilitate presentation and because the differences between the groups were small and consistent (Table 4) and both types of tissue were used in equal numbers.

The following two relationships and the symbols contained in them have been used throughout this paper.

$$[X]_t = [X]_{bd} + [X]_e \cdot V_e + [X]_i \cdot V_i; \qquad (1)$$

where $[X]_t$ = tissue content of ion (m-equiv/kg),
$[X]_{bd}$ = content of ion chemically bound (m-equiv/kg),
$[X]_e$ = extracellular concentration of ion (m-equiv/l. of extracellular fluid),
$[X]_i$ = intracellular concentration of ion (m-equiv/l. of intracellular fluid),
V_e = volume of extracellular fluid (l./kg tissue), and
V_i = volume of intracellular fluid (l./kg tissue).

$$V_i[H_2O]_i + V_e[H_2O]_e = [H_2O]_t; \quad (2)$$

where $[H_2O]_t$ = kg water/kg tissue,
$[H_2O]_i$ = kg water/l. intracellular fluid, and
$[H_2O]_e$ = kg water/l. extracellular fluid.

Other symbols used were Nasp and Clsp for the sodium and chloride spaces respectively. $[Na + K]_t/[H_2O]_t$ refers to the sum of the concentrations of these ions per litre of tissue water.

The extracellular space. The extracellular fluid volume (V_e) is usually estimated by determining the volume of distribution of a substance (such as inulin) which either fails to enter cells or does so at a rate much slower than that at which it penetrates extracellular fluid. Confirmation of this estimate is often sought by comparing it to similar estimates for other such substances or to estimates of the relative area of extracellular space in histological sections. One other possible method is to obtain values for tissue and extracellular concentration of an ion such as chloride and to combine these with estimates of the amount which is bound and of the concentration within cells. Substitution in equation (1) above leaves V_i and V_e as unknowns. V_i can be obtained in terms of V_e by determination of the other quantities in equation (2) and substitution in equation (1). The value for V_e can then be calculated. The weakness in this last approach is that the estimate of intracellular concentration depends on an assumption (e.g. applicability of a Donnan distribution) rather than on direct determination.

In the same types of uterine tissues as those studied in these experiments, the volume of distribution of inulin (250–350 ml./kg) after 3 hr incubation *in vitro* was found in earlier experiments to be smaller than the spaces occupied by predominantly extracellular ions in fresh tissue (Daniel & Daniel, 1957; Daniel, 1958). The chloride, sodium and sulphate spaces were all in the range of 400–700 ml./kg. Moreover, the magnitude of these ion spaces expanded *in vitro*.

Use of the chloride space of fresh tissues as V_e was unacceptable because it led to the calculation of hypertonic concentrations of potassium in cells (> 200 m-equiv/l.) and because it sometimes led to the calculation of negative concentrations of intracellular sodium. Recalculation of the extracellular chloride by assignment of that quantity of chloride to cells which would fit with the assumption that the distribution of this ion was determined by a Donnan potential of 50 mV (inside negative) did not fully eliminate these difficulties, and did not remove the discrepancy between the chloride space and the inulin space (Daniel, 1958). Presumably part of the chloride or part of the potassium or some of both was not ionized, in the sense that it did not contribute to the osmotic pressure or to electrical changes in the tissue space which it occupied. The experiments reported below provided evidence for the first of these possibilities (i.e. chloride partly un-ionized.) Approximately 10 m-equiv/kg of chloride was present in rabbit uteri and 19 m-equiv/kg in pregnant cat uteri after equilibration with sucrose–Ringer solution. In the case of the rabbit uteri recalculation of the chloride space after assignment of chloride to both a bound and a cellular fraction leads to a value of 420 ml./kg. For pregnant cat uteri the figure was 520 ml./kg. Previous experiments have indicated that about 15 m-equiv/kg of sodium does not exchange in isotonic solutions of pure potassium salts and therefore probably is bound (Daniel & Daniel, 1957; H. Singh, unpublished). Correction for bound chloride and sodium leads to the calculation of a potassium concentration of 140–150 m-equiv/l. in the cellular water and to cellular sodium

concentrations of less than 10 m-equiv/l. in fresh uteri. These are credible values. However, the recalculated chloride spaces are, still, larger than the inulin spaces.

Inulin diffuses slowly because of its large molecular volume and may not have penetrated the entire extracellular fluid during the 3 hr period of equilibration. Sucrose also is believed not to penetrate the cellular fluid (Gamble, Robertson, Hannigan, Foster & Fair, 1953; Mulrow, Oestreich & Swan, 1956; White & Rolf, 1957) and usually penetrates a volume slightly larger than the inulin space. It would be expected to diffuse more rapidly than inulin. In experiments, reported below, in which sodium chloride in the medium was replaced by isosmotic quantities of sucrose during recovery after exposure to the cold, the distributions of tissue solids and water were markedly altered after 2 hr (see Tables 1 and 8). This could have resulted from the replacement of the saline-potassium bicarbonate medium in the extracellular space by sucrose solutions containing considerably more solids per unit volume (Table 2). The volumes of the various sucrose solutions required to account for the increments in solids by replacement of the saline solutions were calculated (Table 3).

TABLE 3. Extracellular fluid volumes of uterine tissues exposed to various concentrations of sucrose *in vitro* calculated on the basis of two different assumptions

	Rabbit uterus		Cat uterus	
$[Na]_e$ (mM)	1 (ml./kg)	2 (ml./kg)	1 (ml./kg)	2 (ml./kg)
0	398	—	398	—
20	392	613	425	656
40	430	612	407	722
80	398	620	512	529
100	444	635	398	636
158	—	615	—	615

Assumption (1): that the difference between the solids per kg fresh weight of tissues was due to the difference in the composition of the extracellular fluid when immersed in sucrose-containing solutions and in saline-potassium bicarbonate.

Assumption 2: that the tissue chloride in all these tissues was distributed according to the equation: $[Cl]_t = [Cl]_{bd} + [Cl]_e V_e + [Cl]_i V_i$ when $[Cl]_{bd}$ = the tissue chloride present at $[Cl]_e = 0$, and when $[Cl]_i = [Cl]_e/6{\cdot}5$. This ratio gives a Donnan potential of 50 mV.

These volumes were fairly consistent in both rabbit and cat uteri, averaging about 420 ml./kg in spite of the large maximum errors to be expected in these calculations (7–20%) based on increases in solids of 2·6–7·1 mg in an average 200 mg piece containing 0·08 ml. of extracellular fluid and on weighing to a minimum accuracy of $\pm 0{\cdot}25$ mg). This figure is very close to the corrected value for the chloride space of fresh rabbit uterine tissue and supports the assumption that this is a valid estimate of V_e. The figure is lower than the corrected chloride space in cat uterus (520 ml./kg) but this may be the result of changes in chloride permeability occurring during the prolonged procedures necessary in taking the uterus from pregnant cats whilst saving the animals.

In these same tissues, and in tissues in which choline was substituted for sodium, the slopes of the curves relating concentrations of sodium in the tissue to those in the medium give a crude estimate of V_e. These slopes were from 650 to 735 ml./kg, but were linear only at sodium concentrations greater than 40 m-equiv/l. In media containing sucrose the slopes of curves relating tissue and medium chloride concentrations varied from 650 to 770 ml./kg and were approximately linear. Applications of corrections for bound and for cellular chloride reduced this volume to approximately 625 ml./kg (Table 3). Calculations of a similar nature applied to other tissues after recovery lead to values for V_e near 625 ml./kg in both rabbit and cat uteri. A similar figure (635 ml./kg) is obtained when calculations are made for cat uterine tissue after exposure to cold during the night. For rabbit uteri the

comparable figure is 520 ml./kg, intermediate between the values for fresh tissue and that after recovery.

Clearly, an additional portion of the tissue fluid becomes accessible to sodium and chloride during the incubation *in vitro* of these tissues. Expansion of the volume accessible to sodium and chloride occurs *in vitro* even in experiments omitting the intervening period of metabolic inhibition in the cold (Daniel & Daniel, 1957). Damage to some cells might increase the ion spaces. In calculating intracellular ion concentrations the figures of 420–430 will be used for fresh tissue and the appropriate larger figures for tissues incubated *in vitro*. If expansion of the ion space *in vitro* results from altered permeability in some cells, this procedure involves the assumption that these damaged cells can be treated as equivalent to extracellular fluid.

Figures for cellular concentrations of sodium and potassium have been calculated in some tables by using values for V_e based on this assumption. Two other assumptions were made in calculating these concentrations. They were: (1) the quantity of bound ions was constant and (2) the Donnan potential for chloride did not change. In experiments in which the external potassium concentration was raised this last assumption was probably incorrect. Therefore, the calculations were made assuming either that the extracellular fluid volume (V_e) was unchanged from its value at physiological concentrations of potassium, or that V_e from equation (3) below was such as to fit with the distribution of potassium and chloride according to the same Donnan potential (Conway, 1957; Daniel, 1958):

$$V_e = \frac{[K]_i[Cl]_i - [H_2O]_i^2[K]_e[Cl]_e}{[K]_i[Cl]_e + [K]_e[Cl]_i - 2[H_2O]_i[K]_e[Cl]_e}. \quad (3)$$

In the derivation of this equation potassium and chloride activities in the tissue were assumed equivalent to their concentrations and the concentration of water in cellular and extracellular fluid was taken as unity.

RESULTS

Inhibition by cold and subsequent recovery

Rabbit uterus. Rabbit uterine segments placed in K-free Krebs's solution and kept during the night at 4° C without aeration lost 40–50 m-equiv potassium/kg and gained a similar amount of sodium (Table 4). The total concentration of these univalent cations per litre of tissue was not altered significantly by this procedure but tended to be slightly reduced. Chloride ion penetrated into a slightly greater proportion of the tissue water (about 40–60 ml./kg). This was trivial compared to the marked increase of 250–300 ml./kg in the apparent volume of distribution of sodium. During the over-night interval at low temperature the uterine segments lost solids but gained water.

When returned for 2 hr to oxygenated Krebs–Ringer solution at 37° C, these segments reaccumulated potassium to an extent amounting to more than half the potassium which had been lost (about 25 m-equiv/kg) and expelled a nearly equivalent amount of sodium (about 19 m-equiv/kg). The water which had been taken up was also expelled. The total concentration of these univalent cations per litre of tissue water was increased slightly. Presumably both expulsion of water and net retention of sodium accounted for this slight increase in cation concentration. The increase in

chloride concentration (about 8 m-equiv/kg) was roughly what would be expected to maintain a balance between movements of anions and cations, since gain of K exceeded loss of Na by about 6 m-equiv/kg. The absence of external potassium in the recovery media completely prevented extrusion of sodium as well as reaccumulation of potassium. Extrusion of

TABLE 4. Ions, water and solids in rabbit and cat uterine wall segments

Tissues	Number of experiments	$[K]_t$ (m-equiv/kg)	$[Na]_t$ (m-equiv/kg)	Cl_t (m-equiv/kg)	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Change in [Dry solids] (g/kg fresh weight)	Change in $[H_2O]$ (g/kg fresh weight)
			(*a*) Rabbit uterus				
Fresh control							
Oestrogen	18	67·6	80·9	63·4	172·3	[862]*	—
Oestrogen + progesterone	19	68·3	76·6	61·9	169·1	[856]	—
Cold during night							
Oestrogen	18	20·9	127·2	79·1	168·4	−18	+19
Oestrogen + progesterone	19	22·4	117·8	77·4	160·6	−14	+ 4
Recovery 2 hr at 37° C.							
(1) K-free Krebs's solution							
All	8	21·7	134·2	78·6	175·9	−23	−66
(2) Krebs's solution							
Oestrogen	18	47·3	102·5	88·1	107·4	−19	−13
Oestrogen + progesterone	19	44·0	102·5	85·3	168·1	−12	−10
(3) PO_4-free Krebs's solution							
All	37	46·1	105·1	86·2	172·3	−17	− 2
Cold during night							
All	41	22·7	124·3	85·0	167·6	−17	+44
Recovery 2 hr at 37° C.							
(1) K-free Krebs's solution							
All	39	22·3	124·0	84·8	168·7	−22	−27
			(*b*) Cat uterus				
Fresh control	16	57·2	96·6	90·5	187·8	[820]	—
Cold during night	16	15·6	123·8	100·5	162·1	−31	+161
Recovery 2 hr at 37° C.							
(1) K-free Krebs's solution	8	15·8	124·4	92·3	164·5	−29	+76
(2) PO_4-free Krebs's solution	8	29·1	108·7	101·7	159·7	−38	+191

* The numbers in brackets show $[H_2O]_t$ for the fresh controls.

the water which had been accumulated during the night did occur in the absence of external potassium. This would be compatible with the suggestion that water accumulation results from autolytic changes (as suggested by the losses of solids) which cause the intracellular accumulation of osmotically active intermediates (Conway & Geohegan, 1955). The fact that no further exchanges in sodium and potassium occurred during these 2 hr in warmed K-free medium suggested that the remaining potassium was very difficult to remove from the cell and may have been bound or

confined behind a diffusion barrier such as the mitochondrial membrane (Mudge, 1953).

Table 4 also indicates that a Krebs–Ringer solution prepared without phosphate was just as effective as a recovery medium as a solution containing phosphate. This phosphate-free medium was used subsequently in most studies with metabolic inhibitors because it allowed a number of problems of solubility to be avoided.

Table 4 also shows the characteristic but significant differences in uterine segments pre-treated with progesterone (Daniel & Daniel, 1957). During the night they lost less potassium and gained less sodium and chloride. In addition, they regained less potassium and extruded less sodium during recovery. Apparently progesterone pre-treatment reduced the leakiness of tissues during incubation at 4° C. As a consequence, these tissues started recovery with more cellular potassium and less sodium and hence recovery was less.

Cat uterus. Pregnant cat uterus is like skeletal muscle and unlike the muscle of rabbit uterus in a number of its electrical properties, e.g. excitation by cathodal currents, conducted, all-or-none action potentials (Bozler, 1938; Daniel & Singh, 1958; Daniel, 1960). It is like uterine muscle from non-pregnant rabbits in other properties: excitation by stretch and persistence of action potentials unaltered in amplitude, in configuration or in conduction velocity in media containing one-fifth the normal sodium concentration (Daniel & Singh, 1958; Daniel, 1960; H. Singh, unpublished). Active transport of sodium and potassium in uterine strips from pregnant cats also resembles that in rabbit uterine segments. As is illustrated in Table 4, strips of cat uterus lost potassium and gained sodium after exposure during the night to cold K-free Krebs's solution. A loss of solids and a marked uptake of water also occurred. The gain in sodium was usually less than the loss of potassium, so that the total of these univalent cations per litre of tissue was slightly diminished. This may have resulted from the use of a medium with a sodium concentration some 15–18 m-equiv/l. less than that in cat plasma (Daniel & Daniel, 1957). No extrusion of the accumulated sodium occurred in the absence of external potassium, when these strips were exposed to warm, oxygenated media. Likewise, no further loss of potassium or gain of sodium was found in such tissues. Extrusion of sodium and reaccumulation of potassium did occur when potassium was present in the recovery medium. This recovery was relatively less complete than that occurring in rabbit uterine segments, amounting to a reaccumulation of only 10–20 m-equiv potassium/kg. There was extrusion of a roughly comparable quantity of sodium.

Localization of ion exchanges in rabbit uterine segments

The uterus is composed of several histological cell types inseparably mixed together. In addition, it contains two separate layers which differ in their predominant cellular component, the endometrium and the myometrium. Separation of the layers is difficult to carry out (Daniel, 1958). To guarantee that at least one component should be free from contamination with the other, the longitudinal muscle layer of the myometrium was separated from the remainder of the segment just before the

TABLE 5. Localization of tissue components which actively transport sodium and potassium in rabbit uterus

Tissue components	Procedure	$[K]_t$ (m-equiv/kg)	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$ (m-equiv/kg)	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Nasp –Clsp (ml./kg.)	$[H_2O]_t$ –Clsp (ml./kg)	$[H_2O]_t$ –Nasp (ml./kg)
Myometrium	Control	75·3	96·3	68·8	208·5	26	200	173
	Night in cold K-free Krebs's solution	31·5	149·7	86·5	217·5	354	142	−212
	+120 min in PO_4-free Krebs's solution at 37° C.	67·7	113·2	89·1	216·5	111	122	116
Endometrium + myometrium	Control	67·7	92·2	68·8	187·8	−3	228	236
	Night in cold K-free Krebs's solution	22·7	136·1	88·1	183·2	250	165	−126
	+120 min in PO_4-free Krebs's solution at 37° C.	40·5	115·9	91·9	180·2	108	132	25
Intact	+120 min in PO_4-free Krebs's solution at 37° C.	43·4	109·2	85·8	175·4	122	196	76
After recovery and recording of activity	+120–240 min in PO_4-free Krebs's solution at 37° C.	48·5	102·5	94·1	171·9	−7	125	132

Each value is the mean of 8 determination

final weighing and the two portions analysed separately. Table 5 summarizes the results of such an experiment. The isolated myometrium lost 44 m-equiv potassium/kg and gained 53 m-equiv sodium/kg during the night. The chloride space expanded and calculations indicated that about 7·3 m-equiv chloride/kg had moved into cells. This was approximately enough to account for the uptake of sodium in addition to that exchanged for potassium. After 2 hr in the recovery medium the myometrium had regained 36 m-equiv potassium/kg and expelled a like amount of sodium. Though similar amounts of sodium and potassium were exchanged during the night in the remainder of the uterus, recovery was much less complete 18 m-equiv potassium/kg regained and 20 m-equiv sodium/kg expelled. Chloride concentrations, and derived V_e values, in the myometrium and whole uterus showed no obvious differences before or after recovery. These results strongly suggested that the recovery process occurred chiefly in the myometrium, since its extent is reduced when other tissue components are present.

Physiological functioning of pieces after recovery

The concentrations of water and electrolytes in these segments after 2 hr recovery were similar to those in pieces which had been exposed only to Krebs–Ringer solution in previous experiments in which motility or electrical activity was recorded (Daniel, 1958; Daniel & Singh, 1958; H. Singh, unpublished). Several pieces were therefore taken after exposure during the night to the cold K-free medium, slit along their mesenteric attachments and mounted in the usual fashion in Krebs–Ringer solution for study of electrical and mechanical recording (Daniel & Singh, 1958; H. Singh, unpublished; Daniel, 1960). Within 2 hr after being so prepared spontaneous mechanical activity had begun and typical action potentials were recorded. Although these tissues were slit open so that the endometrial and myometrial surfaces were equally exposed to the recovery medium, and although they were usually left in the medium for 3–5 hr, the extent of recovery of tissue electrolytes (Table 5) was indistinguishable from those reported in Table 4. In view of this result it appears unlikely that relative anoxia caused the failure of active transport in the endometrium. Strips of cat myometrium also recovered normal function after exposure to cold for as long as 48 hr.

Intracellular ion concentrations

The average values for intracellular concentrations of sodium and potassium in the fresh, cold-inhibited, and re-warmed uteri can be calculated (Table 6) by using the values for the corrected chloride spaces as an estimate of the extracellular fluid volume (as discussed under Methods). Calculated concentrations of intracellular chloride have not been included because assumptions involving the distribution of this ion were included in one of the processes whereby V_e was estimated. The values in the table indicate that, when loss of selective permeability in some cells is allowed for by the increments in V_e, the recovery after metabolic inhibition is virtually complete. Presumably the cells with increased leakiness are mainly in the endometrium, since the myometrium recovers nearly completely from metabolic inhibition by low temperature. This conclusion is supported by the complete recovery of contractile activity observed in these tissues.

The results demand the postulation of active extrusion of sodium, since the membrane potentials of these myometrial cells average about 50 mV inside negative (Daniel & Singh, 1958) in fresh tissues. The calculated equilibrium potentials for sodium range from 9 to 75 mV inside positive (Table 6). Thus, sodium extrusion from cells cannot be attributed to the electrochemical gradient. The rise in intracellular sodium concentration

during exposure to cold and subsequent recovery also rule out impermeability as an explanation for the selective exclusion of sodium from cells. The equilibrium potentials for sodium in fresh myometrium vary from +43 to +75 mV. They therefore provide no explanation for the repeated failure to record overshoots larger than 10–15 mV (Woodbury & McIntyre, 1956; Daniel & Singh, 1958).

TABLE 6. Ion distribution and equilibrium potential in uterine muscle

Source of tissue	Treatment	V_e (ml./kg)	$[K]_i$ (m-equiv/l.)	E_K (mV)	$[Na]_i$ (m-equiv/l.)	E_{Na} (mV)
Rabbit	Fresh*	420	139	−91	8·5	+75
	Night in cold†	520	49	−72	96	+ 9
	Recovery in Krebs's solution‡	620	149	−92	14	+60
Cat	Fresh*	430	119	−86	30	+43
	Night in cold†	635	50	−53	93	+10
	Recovery in Krebs's solution‡	635	115	−85	29	+43

The values used in all calculations were rounded-off averages. $[H_2O]_i$ was always assumed to be 988 and $[Na]_{bd}$ was taken as 15. $[H_2O]_e$ was taken as 988 (the value for Krebs's solution) except in fresh tissues.

	$[K]_i$ (m-equiv/kg)	$[Na]_i$ (m-equiv/kg)	$[H_2O]_i$ (g/kg)	$[K]_e$ (m-equiv/l.)	$[Na]_e$ (m-equiv/l.)	$[H_2O]_e$ (g/kg)
* The values for fresh tissues were:						
Rabbit uterus	68	80	860	4·5	145	930
Cat uterus	57	97	820	4·5	155	920
† The values were:						
Rabbit uterus	20	123	880	3·3	137	—
Cat uterus	16	124	860	6·8	137	—
‡ The values were:						
Rabbit uterus	45	104	892	4·6	137	—
Cat uterus	30	109	860	4·6	137	—

The calculated equilibrium potentials for potassium (E_K) in fresh tissue are appreciably more negative than those recorded with micro-electrodes (−85 to −92 versus −50 mV). Even using the largest possible V_i (by assuming that V_e is equivalent to the inulin space), the intracellular potassium concentration is found to be far too high to fit with the measured electrochemical gradient. Three other possibilities might explain the discrepancy. First, there may be a fraction of bound potassium in cells which does not contribute to the electrochemical gradient. Even the assignment of all the potassium remaining after inhibition in the cold to a bound fraction reduces the concentration gradient only to 12 times or more, so that E_K remains at least 66 mV negative inside. Secondly, the measurement of membrane potential may be in error because of damage by the micro-electrodes or because of junction potentials. In this regard it is of interest that metabolically deranged myometrial cells have an E_K much closer to the resting membrane potential and an E_{Na} very near to the over-

shoot usually recorded during action potentials. Finally, active inward transport of potassium may occur. Obviously a decision among the three possibilities (potassium binding, errors in measuring resting potentials, and active potassium transport) cannot be reached on the basis of the present data.

Effect of variation in external sodium concentration on recovery

As mentioned above, the presence of external potassium is required for the extrusion of sodium accumulated during metabolic inhibition. This is characteristic of tissues in which sodium is extruded actively in exchange for intracellular potassium. For some fresh tissues it has been proposed (Shanes, 1958) that potassium accumulation depends upon exclusion of external sodium and therefore upon the concentration of sodium in the medium. If recovery of uterine tissues from inhibition by low temperature depends exclusively on exclusion of external sodium, reduction of external sodium should prevent recovery.

To test this point a simplified recovery medium was used, saline-potassium bicarbonate (Table 1). Recovery in this medium was almost as complete as in Krebs–Ringer solution (compare Tables 7 and 8 to Table 4), despite the absence of calcium. This medium possesses the advantage of allowing alterations in sodium or chloride independently of other ions. In other experiments calcium alone was omitted from the recovery medium without any effect on the process. This probably indicates tight binding of calcium to sites at which it functions rather than the indifference of the membranes of uterine cells to the presence of calcium. In experiments in which electrical and mechanical activity of uteri were recorded in media lacking calcium, repeated washing was necessary to demonstrate that uterine muscle requires calcium for contraction.

There was substantial reaccumulation of potassium in all tissues, despite reduction or absence of external sodium, irrespective of whether choline or sucrose was used as a substitute (Tables 7 and 8). When sucrose was substituted in varying amounts for sodium chloride, potassium reaccumulation was not significantly less than in the unsubstituted media. When choline was substituted for sodium ion, so that the sodium concentration was reduced to less than 80 m-equiv/l., reaccumulation of potassium was diminished. However, only in strips of pregnant cat uterus did reduction of sodium by replacement with choline to levels incompatible with electrical and mechanical activity (0 and 20 m-equiv/l.; Daniel & Singh, 1958) cause any further inhibition of potassium accumulation. In cat uterus replacement of sodium with choline diminishes potassium reaccumulation from 12·1 m-equiv/kg at 40 m-equiv/l. to 2·6 m-equiv/kg when sodium was absent. In general, however, these data support the hypothesis that

TABLE 7. Effect of external sodium concentration on active transport by rabbit uterus

Tissues	$[Na]_o$	Number of expts.	$[K]_t$ (m-equiv/kg)	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$ (m-equiv/kg)	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Changes in: Dry solids (g/kg fresh weight)	Changes in: $[H_2O]_t$ (g/kg fresh weight)	$[Na]_i$ (m-equiv l.)	$[K]_i$ (m-equiv l.)	$[Na+K]_i$ (m-equiv l.)
Choline substituted for sodium in saline-$KHCO_3$ medium											
Oestrogen	0	4	31·2	16·9	112·6	56·2	−13	+16	7	107	114
	20	4	33·5	31·3	116·2	75·1	− 9·5	+ 4	−3	109	—
	40	4	30·5	37·0	112·2	77·2	−13·5	+68	−8	95	—
	80	4	35·1	61·4	109·2	110·6	−16·5	+69	−7	112	—
	100	4	38·6	78·2	108·3	133·9	−14·5	+74	10	132	142
	158	4	37·7	112·4	112·2	171·2	−17	+111	5	124	129
Oestrogen + progesterone	0	4	30·4	29·8	119·9	71·2	−17	−145	60	111	171
	20	4	29·0	34·6	104·5	74·5	−18	−88	28	102	130
	40	4	31·6	48·7	107·6	92·8	−15	−55	31·5	108	139·5
	80	4	32·4	64·9	108·5	114·2	−20·5	−105	0	118	118
	100	4	35·2	82·4	102·1	138·2	−21	−96	20·5	132	152·5
	158	4	40·3	126·5	114·5	191·3	−18	+60	49	142	191
Sucrose substituted for sodium chloride in saline-$KHCO_3$ medium											
All tissues	0	8	34·1	39·0	10·9	90·0	+18	−178	106	137	243
	20	8	42·4	50·3	23·9	114·2	+14	−156	99	171	270
	40	8	41·7	53·9	36·8	115·9	+12	−106	58	162	220
	80	8	43·9	75·7	63·8	142·2	+ 1	−63	44	171	215
	100	8	43·6	88·2	78·9	155·4	− 2	−42	3	169	172
	158	8	39·6	116·6	114·4	170·3	−16·5	+50	11	137	148

See Table 4, part 1, for control values. For calculation of cellular ion concentrations, V_e in choline solutions was the corrected chloride space (see Methods) of the tissues in 158 m-equiv Na/l. V_e was 605 ml./kg in the oestrogen-treated tissues and 623 ml./kg in the progesterone-treated tissues. In sucrose solutions, V_e was 625 ml./kg, from Table 3.

TABLE 8. Effect of external sodium concentration on active transport in strips of pregnant cat uterus*

Tissues	$[Na]_o$	Number of expts.	$[K]_t$ (m-equiv/kg)	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$ (m-equiv/kg)	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Changes in: Dry solids (g/kg fresh weight)	Changes in: $[H_2O]_t$ (g/kg fresh weight)	$[Na]_i$ (m-equiv/l.)	$[K]_i$ (m-equiv/l.)	$[Na+K]_i$ (m-equiv/l.)
Choline substituted for sodium											
All	0	4	22·2	23·4	89·6	54·1	−22	+30	26	61	87
	20	4	28·1	27·9	108·2	66·5	−13	+36	6	80	86
	40	4	31·7	40·3	108·8	84·8	−19	+63	11	90	101
	80	4	35·3	63·2	108·4	116·8	−16·5	+48	15	103	118
	100	4	34·2	77·6	107·0	131·4	−21	+61	25	98	123
	158	4	34·9	112·3	110·7	171·1	−24·5	+120	33	99	131
Sucrose substituted for sodium											
All	0	4	26·6	20·7	18·6	59·8	+ 1·5	−106	27	109	136
	20	4	27·6	32·3	31·9	74·9	0·0	−77	22	112	134
	40	4	27·5	51·3	48·3	96·8	− 6	−35	49	106	155
	80	4	25·5	69·0	65·5	113·5	−10·5	+43	17	95	112
	100	4	27·4	85·5	85·7	133·9	−20	+78	33	100	133
	158	4	29·8	121·5	121·5	176·3	−33	+107	33	115	148

* For calculation of cellular ion concentrations V_e was calculated as in Table 7. For tissues in choline solutions V_e was 544 ml./kg and in sucrose solutions V_e was 625 ml./kg.

internal rather than external sodium ion is critical for reaccumulation of potassium.

In essentially NaCl-free sucrose-Ringer's solution, uterine tissues retain significant quantities of sodium and chloride. Sodium retention has been noted before (Daniel & Daniel, 1957), but proof of chloride retention required a micromethod. In rabbit uterine segments (Table 7) there was more tissue sodium than chloride at all concentrations of these ions in the sucrose-containing media and at a given concentration of external sodium more sodium was retained by the tissues in the sucrose media than in the choline solutions, in confirmation of previous findings (Daniel & Daniel, 1957). In both rabbit and cat uteri (Tables 7 and 8) there was a greater reduction in tissue water in sucrose-Ringer media than in comparable choline-Ringer media ($>$ 200 ml./kg with complete replacement of sodium). In rabbit uterine segments greater reduction in tissue water occurred in choline media if the pieces had been pre-treated with progesterone. In all instances water losses were too large to be explained even by total replacement of tissue fluids by sodium-deficient solutions containing more solids.

To evaluate further the changes in tissue sodium and water the cellular concentrations of potassium and sodium were calculated (see Methods). In choline solutions (Tables 7 and 8), the cellular fluid was deficient in cations. If choline ions, as well as potassium ions, can enter uterine cells in exchange for sodium, the decreased reaccumulation of potassium and the apparent deficiency of cations in cells of these tissues could be explained. The hypotonic cellular cation concentrations suggest that water loss from cells in choline-containing media was secondary to loss of electrolytes.

In pieces of cat uterus exposed to pure choline–Ringer solution the reaccumulation of potassium was significantly decreased as compared with segments exposed to partial choline–Ringer, and in addition the volume of tissue fluid accessible to chloride was significantly diminished. The chloride space in these tissues calculated after corrections for bound and cellular chloride (see Methods) was 448 ml./kg tissue. This value is very close to that calculated for the volume of distribution of sucrose (Table 3) in these experiments and to that of the corrected chloride space of fresh cut uteri. Thus chloride was restricted to a tissue volume equivalent to that accessible to sucrose when there was little or no external sodium. No simple explanation of this observation is entirely satisfactory.

In sucrose solutions pieces of rabbit and cat uterus responded differently. The cellular concentration of potassium was not consistently altered in either type of tissue by reduction of external sodium concentration. However, in rabbit uterus, though not in cat uterus, the cellular concentrations of sodium were calculated to be increased. In addition, the sum of cellular sodium and potassium concentrations was markedly increased. If this result is explained by assuming that rabbit uterine pieces extruded sodium less effectively into sucrose–Ringer solutions, it becomes necessary to make additional postulations to explain the reaccumulation of potassium and the hypertonicity of these cells. A simpler explanation is that the additional cellular sodium is an artifact of the calculations, and that it is really sodium which is outside the myometrial cells and which could not exchange completely or became bound in media with reduced sodium and chloride.

Thus, these experiments provided evidence which strongly supports the independence of active sodium extrusion and of potassium reaccumulation from the concentration of external sodium, but in addition yielded results which are not explicable unless additional assumptions are made.

Recently Whittam & Breuer (1959) have published results similar in some respects to these. They found that potassium retention by the guinea-pig seminal vesicle mucosa in a sucrose medium is identical with that in a complete medium. The small loss of potassium in a choline medium they attribute to choline permeation into the cellular fluid. These authors also noted that in a sodium-free medium potassium retention was independent of metabolic inhibition sufficient to cause marked losses of this ion in the standard medium. They postulated that the retention of potassium under these conditions was dependent on a Donnan equilibrium set up by indiffusible anions. Such a hypothesis would not suffice, however, as an explanation for the sodium extrusion observed in uterine tissues exposed to sodium-poor media.

Elevation of external potassium

According to Conway (1957) potassium and chloride ions freely penetrate the membranes of striated muscle cells and distribute themselves according to a Donnan equilibrium. When potassium chloride is added to the bathing medium, it penetrates into the cell water and at equilibrium raises the concentration of potassium and chloride in the cells without a net uptake of water into the cells.

In a number of experiments on rabbit uterus the potassium concentration of the recovery medium was elevated to 25 m-equiv/l. by addition of potassium chloride 20 m-equiv/l. These experiments are summarized in Table 9 which also contains data analysing the effects of the addition of glucose and bicarbonate to the recovery medium (see below). In four of the series of experiments summarized in Table 9 and identified by an asterisk, saline-bicarbonate medium was unmodified except for variation in potassium concentration. When the average values obtained at 5 and 25 m-equiv potassium/l. are compared, it is obvious that the average tissue concentration of potassium was elevated by 18·7 m-equiv/kg (in terms of the tissue water this was an average elevation of 21·7 m-equiv/l.). Therefore, the extra potassium appeared to have been distributed throughout the tissue water. The average tissue chloride, however, increased only 14·1 m-equiv/kg and the chloride concentration increased only 16·4 m-equiv/l. tissue water. Therefore, the extra chloride was not distributed throughout the tissue water. In fact, the chloride space did not expand significantly relative to tissue water in any of these experiments.

The average tissue sodium concentration was diminished by 1·5 m-equiv/kg in these experiments. The sodium concentration per litre of tissue water was decreased by 1·7 m-equiv so that, taken together with the changes in potassium, there was a net increase in univalent cation of approximately 20 m-equiv/l. Tissue water loss generally tended to accompany elevation in potassium, so that the increased ion concentration probably resulted from osmotically impelled loss of cellular water. Under the experimental conditions used, potassium appeared to enter cells of rabbit uterine segments mainly accompanied by chloride, but also to a smaller extent in exchange for sodium or hydrogen ion and accompanied by an anion other than chloride.

The effect of elevation of external potassium on intracellular ions was analysed by calculations using the corrected chloride space as V_e for each group of tissues exposed to 5 m-equiv potassium/l. and then assuming that V_e was the same in the comparable pieces exposed to 25 m-equiv potassium/l. In every group the potassium concentration in the cells was estimated to increase more than the chloride concentration (19, 42, 26·5 and 16 versus 4, 11, −2, and 11 m-equiv/l. respectively; over-all averages 26 and 6·5 m-equiv/l.).

One other assumption could plausibly be used in estimating V_e; namely that potassium and chloride distribution between cells and medium were determined by the same Donnan potential (see Conway, 1957; Daniel, 1958). Using these values for V_e the over-all average increase in intracellular potassium was calculated to be greater than that of chloride (33 versus 16 m-equiv/l.). However, in two of the four groups (III and IV) the group averages indicated a larger increase in cellular chloride concentration (17 and 24 m-equiv/l.) than in potassium (5 and 7 m-equiv/l.). Given the fact that the total tissue potassium increases more than the tissue chlorides in these two groups, the above values for changes in cellular concentrations could only have occurred if the values of V_e had decreased and those of V_i increased after elevation of the potassium concentration.

In view of the fact that the tissue water concentration was decreased in these two groups at 25 m-equiv K/l. compared to the water concentration at 5 m-equiv K/l. in the same series ((−8)−(+26) = (−34) in III and (+68)−(+91) = (−23) in IV), it is difficult to accept the validity of calculations which indicate an expansion of cellular fluid volume. However, these results illustrate the necessity of independent and accurate measurements of V_e if cellular ion concentrations are to be estimated without ambiguity.

The Donnan ratios obtained in these last calculations averaged 34 in the 5 m-equiv K/l., and decreased to 8 in the 25 m-equiv K/l. These ratios lead to potentials of 94 and 55 mV, respectively, inside negative.

Effects of bicarbonate and glucose

A medium containing only sodium and potassium chloride permits active transport in rabbit uterine segments, though sodium extrusion was less than potassium reaccumulation (Table 9). Potassium reaccumulation improved when bicarbonate and glucose were added to the recovery medium. Sodium extrusion was not similarly improved. Thus, a further significant increase in the concentration of univalent cations in the tissue water occurred in this medium. Additional experiments were carried out to determine whether the glucose or the bicarbonate was responsible for

TABLE 9. Effect of bicarbonate, glucose and potassium chloride on active transport in rabbit uterine segments

Series of expts.	Tissues	Number of expts.	Addition to saline medium (mM)				$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Change in	
			$NaHCO_3$	Glucose	Sucrose	KCl					Dry solids (g/kg fresh weight)	$[H_2O]_t$
I	All	5	0	0	0	0	23·8	123·0	99·7	167·0	− 22	+ 27
		5	0	0	0	5	42·0	116·2	104·6	179·5	− 27	+ 63
		5	0	0	0	25	56·4	118·4	129·9	198·4	− 24	+ 54
		5*	22	49	0	0	20·8	139·3	91·2	183·6	− 22	+ 55
		5	22	49	0	5	49·6	121·3	98·4	194·8	− 19	+ 16
		5	22	49	0	25	66·4	116·4	111·4	209·6	− 21	+ 23
II	All	8	22	0	46	0	20·5	154·4	106·6	196·1	− 22	− 89
		7	22	0	46	5	43·3	126·1	110·6	206·8	− 17	− 15
		8	22	0	46	25	61·6	118·4	125·7	201·7	− 18	− 62
		8*	22	49	0	0	17·1	145·0	98·1	185·6	− 22	− 18
		8	22	49	0	5	44·2	113·4	106·5	179·0	− 22	+ 29
		8	22	49	0	25	66·2	118·1	122·0	211·0	− 22	− 1
III	All	7	0	49	0	0	20·1	127·4	116·3	169·5	− 23	− 40
		8	0	49	0	5	38·8	116·4	122·1	177·7	− 23	+ 19
		8	0	49	0	25	54·1	111·3	139·5	189·9	− 19	+ 4
		8*	22	49	0	0	16·3	147·3	98·6	187·6	− 25	− 39
		8	22	49	0	5	43·6	112·0	105·9	177·5	− 23	+ 26
		8	22	49	0	25	62·2	108·1	118·2	195·5	− 19	− 8
IV	All	8*	22	49	0	0	24·6	127·9	90·4	174·6	− 20	+ 50
		8	22	49	0	5	45·4	111·6	91·3	179·8	− 19	+ 91
		8	22	49	0	25	62·0	108·4	106·4	194·6	− 20	+ 68

* Discussed in text in relation to elevation of potassium concentration.

these differences. Addition of bicarbonate appeared to account for most of the improvement in potassium reaccumulation and the elevation of the concentration of univalent cations relative to tissue water.

Attempts to attain more complete recovery of tissue electrolyte

The recoveries of the tissue concentrations of potassium and of sodium summarized above fell short of the initial levels in the tissue. Elevation of external potassium concentration to 25 m-equiv/l. resulted in attainment of a potassium concentration similar to the control, but the sodium and chloride had then expanded into tissue fluids which did not ordinarily contain these ions. Adrenaline has been found to improve the retention of potassium in these cells (Daniel & Daniel, 1959). In addition, adenosine has been effective in aiding recovery of active transport in stored or starved

TABLE 10. Substances which were ineffective in restoring ionic composition when added to the recovery medium

Substance	Concentrations used
Cocaine*	0·1 g/100 ml.
Adenosine	10 mM
Glutamic acid	10 mM
Physostigmine*	10^{-3} M
Adrenaline*	10 μg/ml.
Calcium ion*	2·5 mM
Sodium versenate†	2 m-equiv/l.
Aldosterone* (free alcohol)	2 μg/ml.
Deoxycorticosterone glucoside ($C_{35}H_{48}O_4$)*	10^{-5} M
Conjugated oestrogens‡	3·14 and 31·4 mg/100 ml.
Progesterone in 1·76% dioxane*	10^{-3} M

* Rabbit uterus only.

† Cat uterus only.

‡ 1 g of water-soluble conjugated oestrogens is equivalent in potency to 0·38 g of sodium oestrone sulphate.

red cells (Kahn & Cohen, 1957; Tosteson & Johnson, 1957*a*, *b*) and glutamate had a similar effect on the glandular tissues of the guinea-pig seminal vesicle (Breuer & Whittam, 1957). Physostigmine has been considered to act as both a stabilizer and labilizer of resting membrane permeabilities (see Shanes, 1958). A wide variety of evidence suggests that, for nerves and skeletal muscle, cocaine and calcium act as stabilizers of resting ion permeabilities (Shanes, 1958; Shanes & Berman, 1959). All these agents were tested in concentrations found effective for other cells as summarized in Table 10. None of these agents significantly improved the reaccumulation of potassium or extrusion of sodium by rabbit uterine segments, and glutamate and adenosine likewise did not cause additional recovery in pieces of cat uterus. Adenosine, in fact, interfered slightly but significantly with potassium reaccumulation in segments of rabbit uterus.

Wilbrandt (1959) has proposed that steroid hormones form part of the carrier system which is involved in active transport. The inhibitory action of cardiac glycosides on active transport has been attributed to their structural similarity to the steroid component of the carrier (Glynn, 1957; Wilbrandt, 1959; Hadju & Leonard, 1959). Ouabain (Daniel & Robinson, 1960) is an effective inhibitor of active transport in rabbit uterine segments. Attempts to influence active transport in red blood cells by steroid hormones such as deoxycorticosterone or aldosterone have been reported to be unsuccessful in one instance (Glynn, 1957) but successful in another (Friedman & Friedman, 1958). The latter authors attributed their success to the use of plasma as a suspending medium. Experiments were therefore run in which the influence of aldosterone-free alcohol and deoxycorticosterone glucoside (see Table 10) on recovery of rabbit uterine segments was determined with both phosphate-free Krebs's solution and plasma as the suspending media. All the animals had received only oestrogen in order to avoid any adrenal steroid-like actions of progesterone (Roberts & Szego, 1953). The plasma was obtained from male rabbits. The effects of conjugated oestrogen and of progesterone on active transport in uterine segments from oestrogen-treated rabbits were also examined, in view of the possibility that these steroids might be selectively involved in the carrier system in these tissues. Recovery in plasma was significantly less than in Krebs's solution. None of these steroids improved recovery, irrespective of the medium used, despite the fact that progesterone pre-treatment interfered with active transport (Table 4). In fact, the data suggested that aldosterone and deoxycorticosterone interfered with active ion transport, as has been found in yeast (Conway & Hingerty, 1953) and in skeletal muscle (Maffii & Bianchi, 1958).

DISCUSSION

Comparisons with active transport in other tissues

The requirements for active extrusion of sodium and reaccumulation of potassium in uterine tissues appear to be the simplest yet reported for mammalian tissues. A simple solution containing only sodium, chloride and potassium was sufficient, the potassium in a concentration (5 m-equiv/l.) comparable to those in extracellular fluid. An appreciable but not remarkable improvement in the reaccumulation of potassium and in the extrusion of sodium was achieved by the use of a complete recovery medium (Krebs–Ringer solution). Active transport in the animal's own plasma was not as good as in the artificial medium. The recovery that was achieved occurred chiefly in the myometrium and not in the endometrium. Following recovery the rabbit uterine tissues were mechanically and

electrically as active as freshly isolated controls. In addition, the ionic concentrations in recovered tissues were similar to those in tissues analysed after simple exposure, to Krebs–Ringer solution for several hours (Daniel, 1958).

In skeletal muscle reduction in external sodium concentration or an increase in external potassium concentration has been found necessary to obtain net sodium extrusion (Desmedt, 1953; Conway, 1957). Neither of these alterations in the recovery medium was required for the demonstration of active transport by uterine muscle. The depression of transport by metabolic inhibitors (Daniel & Robinson, 1960) indicates the energy requirement of this process. No exogenous source of substrate to supply energy for this process was needed during the period of observation. The extensive glycogen stores in these tissues may furnish the necessary source of energy (Daniel & Daniel, 1959).

Mechanisms of potassium reaccumulation in uterine tissue

Under the conditions of those experiments potassium reaccumulation occurred chiefly in exchange for intracellular sodium. Evidence supporting this conclusion was the equivalence of sodium loss and potassium gain, and the dependence of sodium extrusion on the presence of external potassium in the medium. Thus, there appeared to be a coupling of sodium extrusion and potassium reaccumulation in uterine muscle during recovery from inhibition of metabolic activity, as is also the case in skeletal muscle and nerve (Hodgkin & Keynes, 1955; Shanes, 1958).

Sodium extrusion was slightly but not markedly improved by elevation of the level of external potassium to 25 m-equiv/l. by addition of potassium chloride. In such circumstances the resultant changes in potassium and chloride suggested that the additional potassium penetrated throughout the tissue water, but that the additional chloride did not. Part of the potassium which entered the cells unaccompanied by chloride may have been exchanged for cellular sodium. The precision of the measurements and the lack of an independent measure of the extracellular volume did not permit a final decision to be reached in this matter.

Some potassium reaccumulation occurred in the absence of either sodium extrusion or uptake of chloride, when bicarbonate was added to the recovery medium. Either potassium was accompanied by bicarbonate across the cell membrane or exchanged for cellular hydrogen ion (Fenn, Rogers & Ohr, 1958), or else it affected intracellular pH in such a manner as to cause a rise in the number of non-diffusible intracellular anions. In view of the evidence from other tissues (Conway, 1957) that bicarbonate and hydrogen ions penetrate cell membranes in limited quantities, the latter possibility appeared more likely.

Mention should be made of that fraction of tissue potassium (18–22 m-equiv/kg) that is retained even after prolonged inhibition in the cold and subsequent rewarming in the absence of external potassium. It may be retained by binding by tissue anions or by isolation in mitochondria (Mudge, 1953). The Donnan equilibrium set up by non-diffusible tissue anions should not maintain potassium inside the cell indefinitely in the absence of external potassium, but sufficient time for true equilibrium may not have been allowed in these experiments. This residual potassium can be mobilized in part by metabolic inhibitors and by ouabain (Daniel & Robinson, 1960).

Failure to obtain complete recovery

In none of the recovery media was potassium reaccumulation or sodium extrusion sufficient to restore the values to those found in the controls. The tissue water not included in the chloride space (non-chloride space) also remains decreased from the original value. In experiments in which segments of rabbit uteri were divided, the results suggested that endometrial cells were responsible for the lack of complete recovery. Since recovery in plasma was in no way better than in the complete medium, this lack of complete recovery cannot be attributed to the differences in composition of this medium from that of extracellular fluid or plasma. Addition of various agents found effective in improving active transport in other tissues (adenosine, glutamate, adrenaline, physostigmine) had no beneficial effect on recovery of uterine segments. According to Shanes (1958), cocaine (0·1 %) reduces the resting fluxes of potassium and other ions in nerve and possibly in other tissues. It did not improve the recovery process in uterine muscle. Calcium, another agent with similar actions, was likewise without effect. Thus failure to obtain complete recovery was probably not due to excessive passive leakage of sodium inward and potassium outward.

Proposals have been made that steroid hormones may form a portion of the system for ion transport in a variety of tissues (Wilbrandt, 1959). In artificial recovery media they may be missing. However, addition of aldosterone, deoxycorticosterone, oestrogens, or progesterone to the complete recovery medium did not restore the ion concentrations of rabbit uterine tissue to normal levels. Hence, the conclusion must be drawn that there is some other mechanism as yet unknown, which assists towards the maintenance of ionic equilibrium in the endometrium *in vivo*.

SUMMARY

1. Active extrusion of sodium linked to uptake of potassium has been demonstrated in segments of uterus from rabbits pre-treated with ovarian hormones and from pregnant cats. During metabolic inhibition by ex-

posure to cold these segments of tissue lost most of their potassium into a potassium-free Krebs–Ringer solution and gained an approximately equivalent amount of sodium. There also was some uptake of chloride and water. On return to an adequate potassium-containing medium at body temperature the movements of these ions were reversed, but the original ionic composition was not completely regained. However, on recovery, these tissues contracted spontaneously and produced action potentials and were functionally indistinguishable from fresh uterine tissue. Separate analyses of the myometrium suggested that most of the active transport during recovery was confined to this portion of the uterus. Progesterone pre-treatment of rabbits diminished leakage of ions at 4° C and the extent of the recovery achieved subsequently *in vitro*.

2. Active transport of sodium during recovery from metabolic inhibition was prevented by the absence of external potassium, while reaccumulation of potassium was relatively independent of the concentration of external sodium. Sodium extrusion linked to uptake of potassium was therefore the probable mechanism of active ion transport in these tissues. Whether or not a portion of the potassium accumulation in cells was the result of active transport could not be decided.

3. The absences of phosphate and calcium from the recovery medium did not influence the extent to which active transport occurred in rabbit uterine segments. The addition of bicarbonate to a simple unbuffered recovery medium increased the uptake of potassium but this was not accompanied by additional sodium extrusion. Elevation of the concentration of external potassium from 5 to 25 m-equiv/l. by addition of potassium chloride elevated the concentration of potassium by about 20 m-equiv/l. throughout the tissue water, but there was not an equivalent increase in chloride concentration.

4. Various substances were added to the recovery medium to attempt to restore the ionic concentrations to the levels in fresh tissue. Adrenaline, physostigmine, adenosine, glutamic acid, and cocaine were without a significant effect. The addition of aldosterone, deoxycorticosterone, conjugated oestrogens and progesterone likewise had no beneficial effect. The use of plasma as a recovery medium with or without the addition of the mineralocorticoids had a slight inhibitory effect on active transport.

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THE RELATION OF SODIUM SECRETION TO METABOLISM IN ISOLATED SODIUM-RICH UTERINE SEGMENTS

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An extensive literature has grown up indicating that inhibitors of oxidation (N_2, cyanide, azide, barbiturates) or the uncouplers of oxidations to energy-yielding reactions (2, 4-dinitrophenol, azide) interfere with active transport of ions in nerve and striated muscle (see Conway, 1957; Shanes, 1958). Inhibitors of glycolysis, under aerobic conditions, are sometimes effective in inhibiting active transport, provided that the substrate is glucose. However, if pyruvate or lactate is the substrate, glycolysis is bypassed and aerobic metabolism can provide the energy for active transport. Shanes (1958) in a recent review summarized the situation for nerve as follows (p. 103): '(*a*) cessation of aerobic metabolism reveals an ionic leak to sodium and potassium which normally is present—but obscured by the metabolically linked aerobic transport of potassium and sodium against their gradients, (*b*) under anaerobic conditions glycolysis can supply energy, but not rapidly enough in nerve even when glucose is supplied, to achieve adequate metabolic transport'. These statements also appear to apply to muscle (Conway, 1957) to kidney slices (Mudge, 1951, 1953; Whittam & Davies, 1954) and to yeast (Conway, Ryan & Carton, 1954; Conway, 1957).

In some cells, however, anaerobiosis or inhibition of oxidation does not inhibit active transport completely while inhibitors of glycolysis do. This seems to be true of blood cells of certain species (Maizels, 1949, 1951; Tosteson & Johnson, 1957*a*, *b*), but not of others (Maizels, 1954). Some plant cells also can supply energy for active transport in the presence of cyanide or other inhibitors of oxidation (Eppley, 1958*a*, *b*) and others are inhibited by iodoacetate in the dark (Scott & Hayward, 1953). Frog skin can continue active ion transport, though at a reduced rate, during anaerobiosis (Leaf & Renshaw, 1957).

Active transport during recovery of rabbit and cat uterine segments from the inhibition of metabolism by low temperature has been studied (Daniel & Robinson, 1960) and appears to be a coupled exchange of cellular sodium for extracellular potassium. This study was undertaken to determine the dependence of active transport in uterine muscle on metabolism.

Inhibitors both of glycolysis (fluoride, iodoacetate) and of oxidation (nitrogen, cyanide, azide, pentobarbital, antimycin, malonate) have been tested for their effect on active transport.

The former were found effective but the latter were largely ineffective, with the exception of pentobarbital. 2,4-Dinitrophenol was effective in preventing potassium reaccumulation and sodium extrusion, as was the cardiac glycoside ouabain. Because of the possibility that apparent inhibition of active transport might result from an increase in the leakage of sodium and potassium down their electrochemical gradients, an attempt was made to diminish such leakage during metabolic inhibition in some experiments by the use of cocaine (Shanes, 1958). Interpretation of the results was limited by the paucity of knowledge concerning the normal metabolism of smooth muscle and the effects of inhibitors thereon.

METHODS

The experimental procedures for preparation and analyses of uterine tissues and the demonstration of active transport have been described in the previous paper (Daniel & Robinson, 1960), together with the composition of the solutions used and the methods of handling the data obtained. The tissues were suspended in 5 ml. of solution in a test-tube for study. The aerating mixtures were bubbled into the solution at the bottom of the tube and escaped through a small opening (2 mm diam.) in a cork at the tip of the tube. This arrangement was intended to guarantee that the atmosphere over the solution was not diluted by room air.

When inhibitors were added as the sodium salt (NaCN, NaN_3, Na pentobarbital, Na malonate, NaF) approximate isomolarity was maintained by removal of an equivalent amount of sodium chloride. This of course resulted in a decrease of the tissue chloride concentrations in these instances, but this was taken into account in calculating chloride space. Ouabain was added to the cold, potassium-free Krebs's solution 3 hr before removal of the tissues to be studied, in order to allow time for its full effect to be achieved (Glynn, 1957). It was also present in the same concentration in the recovery medium.

RESULTS

Rabbit uterine segments

Inhibitors of oxidation (Table 1). Replacement of the oxygen in the aerating gas mixture with nitrogen only slightly depresses extrusion of sodium and accumulation of potassium of rabbit uterine segments. Compared with similar segments exposed to a similar gas mixture in the absence of potassium, sodium extrusion was 31·7 m-equiv/kg in oxygen and 28·9 m-equiv/kg in nitrogen, an insignificant difference. Potassium accumulation was 23·9 m-equiv/kg in oxygen and 19·3 m-equiv/kg in nitrogen, a small but significant difference. The excess of sodium extrusion over potassium reaccumulation results from the occurrence of additional uptake of sodium without additional loss of potassium or gain of water and chloride in uterine segments during the incubation in potassium-free

medium (Table 3, Daniel & Robinson, 1960). In nitrogen the water which had accumulated during metabolic inhibition in the cold was not expelled, presumably because of the continued presence of organic osmotically active particles.

Cyanide. Cyanide inhibits the enzymic activity of cytochrome oxidase in most tissues (Keilin & Hartree, 1939; Robbie, 1949; Chance, 1956). Numerous attempts to inhibit active sodium–potassium exchange by using cyanide in rabbit uterus segments have failed. Table 1 contains the

TABLE 1. Effects of inhibitors of respiration on active transport in rabbit uterine segments

Experimental procedures	No. of expts.	$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Nasp –Clsp (ml./kg)	Change in Dry solids (g/kg fresh weight)	Change in $[H_2O]_t$
I. 95% O_2+5% CO_2 Krebs–Ringer solution	37	45·6	102·5	86·7	169·3	47	−15·5	−11
K-free Krebs's solution	8	21·7	134·2	78·6	175·9	348	−23	−66
95% N_2+5% CO_2 Krebs–Ringer solution	8	37·9	106·4	86·3	164·5	84	−18	+30
K-free Krebs's solution	8	18·6	135·5	82·1	175·7	330	−16	+46
II. PO_4-free Krebs's solution*	7	40·9	102·6	87·6	163·0	46	−24	+70
+NaCN 10^{-4} M	7	38·1	100·2	78·5	158·2	114	−23	+95
+NaCN 10^{-3} M	8	37·1	105·1	85·4	161·2	77	−26	+81
+NaCN 10^{-2} M	14	35·8	109·3	82·9	165·0	75	−23	+73
III. PO_4-free Krebs's solution	8	45·2	111·2	85·3	178·8	105	−19	−30
+Na azide 2×10^{-4} M	8	47·6	115·3	90·4	186·6	115	−16	−27
+Na azide 2×10^{-3} M	8	42·7	116·9	85·2	181·1	158	−24	+50
+NaCN 10^{-2} M	8	38·6	117·6	86·8	177·9	101	−21	+28
+C_2H_5OH† 8·85 g/100 ml.	8	21·3	148·1	100·1	192·8	277	−22	+26
IV. PO_4-free Krebs's solution	8	44·7	103·4	85·2	168·1	71	−20	+18
+C_2H_5OH 0·88 g/100 ml.	8	45·0	100·2	88·1	164·8	25	−20	+20
+C_2H_5OH 0·88 g/100 ml. +Antimycin A 10^{-5} M	8	40·0	107·3	87·4	166·1	82	−26	+87
V. PO_4-free Krebs's solution	8	40·6	97·7	93·6	157·1	−38	−22	+26·5
+Na pentobarbital 10^{-3} M	16	41·8	107·0	89·9	168·9	48	−23	+74
+Na pentobarbital 10^{-2} M	16	9·6	134·8	97·9	161·9	130	−34	+153

* NaCN 10^{-5} M was without effect.

† Antimycin A 10^{-5} M may have had a slight effect on addition to the alcohol in some experiments using this concentration.

results from one such experiment. At very high concentrations of cyanide (10^{-2}M) there was a small but significant decrease in potassium and gain in sodium concentrations of the segments compared with untreated controls. The difference in potassium concentration was 5·1 m-equiv/kg and in sodium concentration 6·7 m-equiv/kg. The effect of cyanide in high concentration was therefore of the same order of magnitude as anoxia. An increase in sodium space relative to chloride at 10^{-4}M cyanide resulted from an abnormally low tissue chloride concentration, which was not found in a number of other experiments at similar and lower cyanide concentrations.

Azide. Because of the results with anoxia and cyanide, it was thought desirable to extend these experiments by including another inhibitor acting in the cytochrome system. Azide is considered to act at the same site as cyanide to inhibit cytochrome c oxidase, but may also act as an uncoupler of phosphorylations (McElroy, 1947; Lehninger, Wadkins, Cooper, Devlin & Gamble, 1958). Like cyanide, it was only weakly effective in inhibiting active ion transport in rabbit uterine segments in concentrations (2×10^{-4}–2×10^{-3}M) which were effective in inhibiting respiration and ion transport in other tissues (Hurlburt, 1958). As with cyanide, high concentrations of azide inhibited the extrusion of the water which had accumulated during the night in the cold.

Antimycin A. Antimycin is an antibiotic substance whose site of action has been shown to be on the transfer of electrons between cytochrome b and cytochrome c in a number of tissues (Strong, 1956; Chance, 1956; Lehninger *et al.* 1958). A small quantity of ethanol (0·88%) was added to dissolve the antimycin and had no effect on active transport. Antimycin (10^{-5}M), like anoxia, cyanide and azide, had only a small, though significant, depressant effect on active sodium–potassium exchange in these tissues.

Na pentobarbital. Barbiturates act to inhibit oxygen consumption either by preventing reduction of flavoprotein (Chance, 1956), or of cytochrome b which accepts electrons from this compound (Quastel, 1943; Grieg, 1946). Pentobarbital has recently been shown to inhibit oxygen consumption of heart muscle by 50% at a concentration of 10^{-3}, and completely at 10^{-2}M (G. Drummond, personal communication). Its effects on action potentials of striated muscle (8×10^{-4}M) have been interpreted as indicating inhibition of active transport (Macfarlane & Meares, 1958). In contrast to other inhibitors of oxidation, pentobarbital (10^{-2}M) completely inhibits active ion transport in rabbit uterine segments. A lower concentration (10^{-3}M) was completely ineffective. Pentobarbital, in fact, appeared to do more than inhibit active ion transport. In the previous paper (Daniel & Robinson, 1960) it was shown that a concentration averaging 20 m-equiv potassium/kg was usually maintained in these

tissues despite metabolic inhibition by cold in potassium-free solutions, and that this potassium was not lost in rewarming. Pentobarbital (10^{-2}M) mobilized that potassium and reduced it by half. It also caused a significant uptake of water and chloride.

Ethyl alcohol inhibits oxidations in concentrations of less than 1M (Beer & Quastel, 1958). A concentration of 8·85 g/100 ml. (about 2M) completely inhibited active ion transport in rabbit uterine segments.

Effects of inhibitors of oxidations on chloride and water distribution. In the strips treated with higher concentrations of cyanide (10^{-2}M) and pentobarbital (10^{-2}M) and with ethanol (8·85%) the chloride space expanded relative to tissue water so that the 'non-chloride space' was smaller. In the case of cyanide this was equivalent to the expansion of the sodium space, so that no increase in sodium space relative to the chloride space

TABLE 2. Effect of 2,4-dinitrophenol (DNP) and sodium malonate on active transport in rabbit uterine segments

Experimental procedures	No. of expts.	$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Nasp −Clsp (ml./kg)	Change in: Dry solids (g/kg fresh weight)	Change in: $[H_2O]_t$ (g/kg fresh weight)
I. PO_4-free Krebs's solution	28	46·1	105·1	186·2	172·3	78	−17	− 2
+DNP $2·5 \times 10^{-5}$M	8	40·5	109·4	89·2	171·4	85	−16	+34
+DNP $2·5 \times 10^{-4}$M	8	31·6	113·8	89·2	164·7	113	−20	+76
+DNP $2·5 \times 10^{-3}$M	8	23·3	116·8	89·5	159·1	108	−21	+79
II. PO_4-free Kreba's solution								
+malonate 5×10^{-3}M	4	40·7	101·1	76·5	161·2	100	−22	+46
+malonate 5×10^{-2}M	4	40·1	99·4	47·9	158·6	88	−22·5	+43

occurred. Sufficient water usually accompanied the sodium and chloride to maintain a constant concentration of the measured univalent cations per litre of tissue water. In other words, cyanide, pentobarbital, and alcohol caused sodium and chloride and water to move into cells and to occupy slightly larger portions of the tissue volume at isotonic concentrations, thereby diluting the normal cellular constituents (e.g. potassium) (see Mudge, 1951, 1953; Whittam, 1956; Leaf, 1956 for a discussion of the intricacies of such shifts). Both active ion exchange and prevention of isotonic swelling require activity of a sodium-extrusion mechanism and therefore depend upon metabolism. In the cases of pentobarbital and alcohol there was an additional movement of sodium into cells undoubtedly in exchange for potassium.

2,4-*Dinitrophenol.* The mechanism of action of dinitrophenol (DNP) has been extensively studied in other tissues (Tables 2 and 5) and shown to consist of the uncoupling of phosphorylations from oxidations (Loomis & Lipmann, 1948, 1949). The precise site of its action in the mitochondria is

not yet settled (Lehninger *et al.* 1958). In concentrations increasing from $2{\cdot}5 \times 10^{-5}$M to $2{\cdot}5 \times 10^{-3}$M it causes partial to complete inhibition of active potassium reaccumulation in rabbit uterine segments. Its inhibitory effect on sodium extrusion, though definite, is much less. Prevention of the uptake of 22·8 m-equiv potassium/kg was not matched by the retention of sodium, which amounted to only 11·7 m-equiv/kg. Water which had accumulated during the night was not removed from DNP-treated cells and probably increased. As a consequence, tissues exposed to DNP have a lower concentration of these univalent cations per litre of tissue water.

TABLE 3. Effect of inhibitors of glycolysis and of lowered temperature on active transport in rabbit uterine segments

Experimental procedures	No. of expts.	$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Nasp –Clsp (ml./kg)	Change in Dry solids (g/kg fresh weight)	Change in $[H_2O]_t$
I. PO_4-free Krebs's solution	28	46·1	105·1	86·2	172·3	78	−17	− 2
+iodoacetate $1{\cdot}7 \times 10^{-4}$M	8	39·7	106·2	86·5	164·9	86	−22	+41
+iodoacetate $5{\cdot}3 \times 10^{-4}$M	8	36·3	113·7	91·3	169·8	110	−20	+17
+iodoacetate $1{\cdot}7 \times 10^{-3}$M	8	25·5	122·6	93·8	164·9	144	−24	+50
II. PO_4-free Krebs's solution	8	39·9	104·2	88·6	165·1	51	−19	+37
Saline-$KHCO_3$ medium	8	39·9	122·2	126·8	185·3	−29	−19	+28
+NaF 10^{-2}M	8	21·6	139·1	102·6	186·6	187	−6	−109
+NaF 5×10^{-2}M	8	7·8	156·8	89·6	187·4	166	−23	+55
III. PO_4-free Krebs's solution	8	51·7	108·4	88·3	182·2	83	−17	+2
Saline-$KHCO_3$ medium	8	45·4	120·9	113·8	189·5	45	− 5	− 5
+NaF 5×10^{-3}M	8	43·7	124·4	97·5	198·0	150	+10	−195
IV. PO_4-free Krebs's solution								
37° C	8	44·7	103·4	85·2	168·1	71	−20	+18
24° C	8	43·9	99·4	82·3	162·7	65	−19·5	+48
4° C	8	22·0	123·0	88·1	164·2	190	−21	+70

These results suggest that dinitrophenol produces alterations in metabolism which permit some sodium to be extruded from uterine cells without any compensatory uptake of potassium or loss of water. In other experiments the difference between the action of DNP on potassium reaccumulation and sodium and water extrusion were not so marked (Table 5). None the less, the possibility must be considered that during DNP inhibition sodium can be extruded to some degree accompanied by a cellular anion or that the potassium accumulated in exchange for sodium leaks out accompanied by such an anion.

Malonate is a competitive inhibitor of succinic dehydrogenase in the tricarboxylic acid cycle (Quastel & Wheatley, 1931), and concentrations

of 10^{-2} to 10^{-1}M inhibited oxygen consumption in heart slices (Miller & Olson, 1954). Malonate (5×10^{-3}–5×10^{-2}M) diminished potassium reaccumulation to a slight and insignificant extent and did not influence sodium extrusion (Table 2). This result cannot be evaluated in the absence of proof that malonate penetrated rabbit uterine cells. However, Covin & Berman (1956) have presented evidence that similar concentrations of malonate had effects on intact heart muscle cells which implied its penetration through the cell membrane.

Inhibitors of glycolysis

Mono-iodoacetate. Iodoacetate has been shown by Green, Needham & Dewan (1937) to inhibit phosphoglyceraldehyde dehydrogenase and thereby to prevent glycolysis. Higher concentrations may inhibit other sulphydryl enzymes (Dixon, 1937) but concentrations of 10^{-4} and 10^{-3}M had little or no effect on oxygen consumption of heart slices (Miller & Olson, 1954). In concentrations ranging from $1{\cdot}7 \times 10^{-4}$ to 10^{-3}M it produced from slight to nearly complete inhibition of active sodium–potassium exchange in rabbit uterine segments. The reduction in potassium accumulation (20·6 m-equiv/kg) was nearly equivalent to the diminution in sodium extrusion (17·5 m-equiv/kg). Closer examination of the data reveals that there was a clear-cut and significant elevation in the chloride concentration with increasing concentrations of iodoacetate. This increase was nearly accounted for if the fluid which moved into cells under the influence of iodoacetate was similar in composition to the Krebs–Ringer solution. The increase in sodium caused by uptake of fluid in $1{\cdot}7 \times 10^{-3}$M iodoacetate (6·8 m-equiv/kg fresh weight or 6·7 m-equiv/kg final weight) did not account for the increase in sodium concentration. As with pentobarbital and with ethyl alcohol, the reduction of potassium accumulation apparently was caused in part by replacement of cellular fluid by isotonic Ringer's solution and in part by inhibition of exchange with cellular sodium.

Sodium fluoride in concentrations from 2×10^{-3} to 5×10^{-2}M inhibits glycolysis and active transport in a number of red cells (Maizels, 1951; Tosteson & Johnson, 1957*a*; Eckel, 1958*a*, *b*). However, similar concentrations inhibit oxygen consumption as well as ion transport in kidney slices (Mudge, 1951). In studying its inhibitory action on active transport a medium free from divalent ions was used (saline-potassium bicarbonate, Daniel & Robinson, 1960). Active transport occurred in this medium, but was accompanied by water or by sodium. Concentrations from 5×10^{-3}M to 10^{-2}M inhibit active ion transport in rabbit uterine segments. Concentrations of 5×10^{-2}M mobilized additional tissue potassium, as did pentobarbital (see p. 448). There was no definite evidence that fluoride,

unlike the other metabolic inhibitors, caused an uptake of fluid similar in composition to the bathing medium, since the chloride space was not consistently expanded relative to the tissue-water concentration and since there was no clear evidence of uptake of water. However, expansion of the chloride space had already occurred in saline-$KHCO_3$ medium in the absence of fluoride, so that interpretation is difficult.

Effect of variations in temperature. Reduction of temperature might be expected to hinder recovery of ion gradients due to active transport by interfering with the supply of metabolic energy. However, reduction of temperature from 37° C to room temperature, 24° C, did not influence the extent of recovery of active sodium–potassium exchange in these tissues (Table 3). As demonstrated for yeast (Bruce & Stannard, 1958) this could

TABLE 4. Effect of ouabain on active transport on rabbit uterine segments

Experimental procedures	No. of expts.	$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Nasp −Clsp (ml./kg)	Change in Dry solids (g/kg fresh	$[H_2O]_t$ weight)
I. PO_4-free Krebs's solution	8	51·9	110·5	102·0	185·3	−18	−21	+12
+ ouabain 10^{-7} M	8	45·2	115·1	99·9	183·7	23	−16	−15
II. PO_4-free Krebs's solution	4	38·4	107·2	85·7	164·9	90	−29	+102
+ ouabain 10^{-6} M	8	22·0	122·8	83·6	164·9	225	−23	+22
III. PO_4-free Krebs's solution	16	45·5	106·4	88·8	173·1	64	−19	+ 6
+ ouabain 10^{-5} M	16	13·8	132·8	94·1	168·9	215	−19	− 1

result from the fact that passive movements of sodium and potassium down their gradients may also be slowed at reduced temperature and compensate for any decrease in the rate of active transport. Further reduction of the temperature to 4° C, however, completely prevented active transport, as was to be expected.

Ouabain and active transport. The cardiac glycosides have been shown to inhibit active sodium–potassium exchanges in a variety of tissues, striate muscle, cardiac muscle and red blood cells (Schatzman, 1953; Schatzman & Witt, 1954; Kahn & Acheson, 1955; Schreiber, 1956; Glynn, 1957; Rayner & Weatherall, 1957; Vick & Kahn, 1957). They appear to act directly on the ion-exchange mechanism rather than on metabolism (Schatzman, 1953; Glynn, 1957; Whittam, 1957). As is demonstrated by the data in Table 4, they also inhibit this process in rabbit uterine segments. Ouabain in concentration of from 10^{-7} to 10^{-6} M produced slight to complete inhibition of the process. A concentration of 10^{-5} M ouabain, like pentobarbital and fluoride in high concentration appeared to mobilize additional potassium, so that less than the usual 20 m-equiv/kg was retained. Ouabain even in this high concentration does not cause a signi-

ficant uptake of water or chloride, so that its actions seem to be primarily by inhibition of sodium–potassium exchange without any accompanying swelling of cells by uptake of the isotonic bathing medium. Glynn (1957) has shown that cardiac glycosides inhibit passive fluxes as well as active, and failure to take up sodium isotonically may be secondary to this effect of ouabain.

TABLE 5. Effect of cocaine on inhibition of active transport in rabbit uterine segments

Experimental procedures	No. of expts.	$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/l.)	Nasp –Clsp (ml./kg)	Change in Dry solids (g/kg fresh	Change in $[H_2O]_t$ weight)
I. PO_4-free Krebs's solution	8	51·9	110·5	102·0	185·3	−18	−21	+12
+cocaine HCl 0·1g/100 ml.	8	54·3	115·4	105·2	195·7	−1	−13	−43
+DNP $2·5 \times 10^{-3}$M	8	27·3	133·0	104·7	180·4	101	−26	+97
+cocaine HCl 0·1g/100 ml.+ DNP $2·5 \times 10^{-3}$M	16	34·9	124·3	108·3	180·1	0	−28	+87
II. PO_4-free Krebs's solution	8	51·7	108·4	88·3	182·2	83	−17	+ 2
+cocaine HCl 0·1g/100 ml.	8	46·2	108·0	91·3	175·7	56	−17	+10
+iodoacetic acid $1·7 \times 10^{-3}$M	8	26·8	131·0	98·6	173·2	165	27	+86
+cocaine HCl 0·1g/100 ml.+ iodoacetic acid $1·7 \times 10^{-3}$M	8	22·1	121·4	93·1	164·3	119	−26	+115

Effect of cocaine on inhibition of active transport. The ionic composition of any tissue is probably the resultant of two opposing processes: active transport against ionic gradients and passive leakage of ions down these gradients (Shanes, 1958). Apparent inhibition of active transport could be produced by substances which markedly increase the rate of passive leakage of these ions so that they leached out as fast as they were transported into cells. Cocaine has been reported (Shanes, 1958; Shanes & Berman, 1959) to diminish the rate of passive leakage of ions at least in nerves. We have shown (Daniel & Robinson, 1960) that cocaine has little effect on the ionic composition of rabbit uterine segments in the absence of metabolic inhibition. As is shown by data assembled in Table 5, cocaine 0·1 g/100 ml. in part prevented the effect of DNP on the recovery of ionic gradients by rabbit uterine segments. Cocaine did not prevent the uptake of water and chloride which occurs in the presence of DNP. The possibility existed that cocaine prevented all effects of DNP except swelling. About 75 ml. more water/kg fresh weight was taken up in tissues exposed to both DNP and cocaine than by the untreated control pieces. Swelling of cells by this volume of a fluid containing sodium at the concentration in the

bath medium would cause an uptake of 10·3 m-equiv/kg fresh weight of sodium. In terms of final weight, this value becomes 9·7 m-equiv/kg. Therefore swelling would account for most of the increase in tissue sodium (13·8 m-equiv/kg) over the amount in the untreated controls. The increase in weight, compared with controls, of 68 g/kg fresh weight would reduce the potassium concentration by 3·3 m-equiv/kg, and failure to exchange for the sodium in excess of that from swelling (13·8–9·7 m-equiv/kg) would reduce potassium concentration by 4·1 m-equiv/kg, leaving 9·6 m-equiv/kg of the reduction in potassium concentration unaccounted for by either mechanism. Qualitatively similar conclusions are reached if comparison is made with the cocaine-treated control except that all the sodium uptake could be explained by swelling. In the absence of cocaine the reduction by DNP of potassium concentration of 24·6 m-equiv/kg was accounted for in part by swelling (3·8 m-equiv/kg) and by failure to exchange for sodium (11·7 m-equiv/kg), and 9·1 m-equiv/kg was unaccountable. Thus cocaine appeared partially to restore the active exchange of external potassium for internal sodium inhibited by DNP without influencing other actions of DNP (swelling and loss of potassium by other mechanisms). Apparent restoration of active sodium–potassium exchange would result from decreased passive leakage of potassium since this leakage would be accompanied by uptake of sodium. Again these findings raise the possibility, as discussed above, that DNP alters the cells of rabbit uterine segments so that the linkage between sodium extrusion and potassium accumulation is disrupted.

Iodoacetate. With iodoacetate a somewhat different effect was produced on addition of cocaine to the bath medium. The inhibition of potassium reaccumulation produced by iodoacetate was not reversed by cocaine, but the inhibition of sodium extrusion and uptake of chloride was partially prevented. Cocaine did not decrease the uptake of water induced by iodoacetate. Consequently, the sum of the measured univalent cations per litre tissue water was diminished. This result is difficult to interpret, but the suggestion can be made that under the influence of cocaine and iodoacetate some intermediary metabolites accumulate in the cells and prevent the entrance of sodium and chloride as ion pairs.

Cat uterine segments

The effects of most of the same inhibitors on active transport in strips of uterus from pregnant cats have been surveyed and the results summarized in Table 6. The results obtained are qualitatively similar to those obtained with segments of rabbit uterus. Pentobarbital, DNP, iodoacetate, fluoride and ouabain are effective in preventing sodium extrusion and potassium reaccumulation. Inhibition of potassium reaccumulation by DNP was

more marked than inhibition of sodium extrusion, in conformity with the results obtained with segments of rabbit uterus. Azide and malonate were ineffective. When cyanide was used as the inhibitor, the results were variable. In experiments II and III (Table 6) there was evidence that 10^{-3}–10^{-2} M cyanide inhibited potassium reaccumulation but extrusion of sodium was not significantly decreased. In experiment IV cyanide in these same concentrations was ineffective. At present no explanation can be advanced for this discrepancy.

TABLE 6. Effect of metabolic inhibitors and ouabain on active transport in strips of pregnant cat uterus

Experimental procedures	No. of expts.	$[K]_t$	$[Na]_t$ (m-equiv/kg)	$[Cl]_t$	$\frac{[K+Na]_t}{[H_2O]_t}$ (m-equiv/kg)	Nasp –Clsp (ml./kg)	Change in Dry solids (g/kg fresh	Change in $[H_2O]_t$ weight)
I. PO_4-free Krebs's solution	4	34·1	98·1	95·0	154·7	−46	−17·5	+27
+DNP $2·5 \times 10^{-4}$ M	8	24·8	100·0	92·1	146·6	−8	−19·5	−44·5
+iodoacetic acid $1·7 \times 10^{-3}$ M	8	23·9	104·5	90·7	151·4	−2	−13	−45
+Na malonate 5×10^{-2} M	8	34·0	94·5	55·2	150·0	−82	−20	−21
II. PO_4-free Krebs's solution	4	28·0	107·1	99·7	156·4	−18	−40	+181
+ouabain 10^{-5} M	8	5·2	125·2	105·4	149·7	68	−50	+195
+NaCN $8·3 \times 10^{-3}$ M	8	23·7	111·9	100·9	157·6	2	−39	+120
III. PO_4-free Krebs's solution	4	30·1	109·8	103·7	163·3	−31	−35	+200
+NaCN 10^{-3} M	4	19·6	108·6	106·3	148·7	−66	−41	+215·5
+NaCN 10^{-2} M	4	15·6	113·6	97·4	150·6	−20	−36	+198
+Na pento-barbital 10^{-2} M	4	7·6	124·5	98·9	153·3	46	−39	+243
Saline-$KHCO_3$	4	21·3	130·1	138·5	174·1	−53	−47·5	+293·5
+NaF 5×10^{-3} M	4	14·6	134·1	132·4	173·8	−18	−27	+133
V. PO_4-free Krebs's solution	4	24·5	110·3	98·9	154·3	12	−53	+130·5
+NaCN 10^{-3} M	8	25·4	110·6	97·2	156·6	21	−47	+204
+NaCN 10^{-2} M	8	24·6	105·1	89·3	150·4	−12	−41·5	+56
+Na azide 2×10^{-3} M	8	23·1	106·2	95·9	149·7	−3	−43·5	+37·3

Another difference from the results for rabbit uterus was the failure of any of the metabolic inhibitors to cause accumulation of additional water. Moreover, none cause a significant uptake of chloride or expansion of the chloride space relative to the tissue water. Therefore there was no indication of any uptake of isotonic bath fluid as a result of metabolic inhibition. This may have resulted from the fact that large quantities of this fluid and considerable swelling were present even in the control tissues, and further swelling may have been impossible owing to mechanical pressures exerted by the tissues.

DISCUSSION

The inhibitors used in this study were selected with the purpose in mind of surveying the effects on active transport of inhibition at various points in the metabolic pathways from glucose to carbon dioxide and water. The transfer of conclusions as to the sites of action in other systems to uterine tissues can be hazardous in the absence of direct evidence.

There was, however, a certain amount of consistency in the results. Inhibitors of glycolysis (iodoacetate and fluoride) were both effective inhibitors of active ion transport in both rabbit and cat uterus under aerobic conditions. Inhibition at or near the end of the chain of electron transfer through the cytochrome system was ineffective (N_2, cyanide, azide, antimycin A). Malonate acting in the Krebs's cycle was ineffective but it may not have penetrated the cell membrane, and in any case acts at the substrate level of oxidation. Obvious inconsistencies arise in relation to the actions of dinitrophenol, ethyl alcohol and pentobarbital. Pentobarbital probably inhibits the same site as amytal (Chance, 1956), which is probably before entrance of electrons into the cytochrome system. The site of action of alcohol in oxidative metabolism is not known, but it is ineffective in mitochondrial preparations in concentrations effective in slices (Beer & Quastel, 1958). DNP is thought to act in mitochondria on reactions linking phosphorylation to electron transfer (Lehninger *et al.* 1958). However, at least a part of the action of DNP appeared to be due to increased potassium leakage and there was evidence that it inhibited potassium accumulation and uptake of isotonic fluid to a greater extent than it inhibited sodium extrusion. The action of pentobarbital on phosphorylations is not striking and the effect of cocaine on its inhibitory action was not studied.

In many other respects, these results are parallel to those obtained with similar inhibitors during recovery in cold-stored human erythrocytes (Maizels, 1951). In these cells, however, dinitrophenol was ineffective and barbiturates were not tested. In human red cells, which are without a nucleus, oxygen consumption is sluggish and aerobic glycolysis occurs. Significant lactate accumulation has recently been demonstrated to occur in rat uterus metabolizing glucose in oxygen (Gautheron & Matray, 1958). Lactate accumulation was greatest in Krebs-bicarbonate medium and the respiratory quotient was slightly less than 1·0. Glucose increased both lactate accumulation and oxygen consumption. Thus uterine metabolism appears to resemble that of red cells in lacking efficient oxidative pathways and active transport may require energy from glycolysis. Studies of the effect of these inhibitors on oxygen consumption and lactate accumulation in rabbit and cat uterus will be required to determine the accuracy of this

hypothesis. Mention should be made of the possibility that lactate ion may contribute to the ionic balance of smooth muscle and to its electrical properties as a consequence.

If uterine muscle does depend on glycolytic energy for active transport, the mechanism of the inhibitory actions of dinitrophenol and pentobarbital on active transport will require elucidation. DNP, in concentrations similar to those which inhibit active transport in uterus, first stimulated and then inhibited the electrical and mechanical activity of the guinea-pig's taenia coli (Born & Bülbring, 1955; Bülbring & Lüllman, 1957). Resting potentials were diminished during the excitatory phase of the action of DNP and then were restored during inhibition (Burnstock, 1958). During the phase of inhibition the duration of spike activity produced by acetylcholine was diminished and this was interpreted as being due to interference with active transport. This interpretation was supported by the previous report of Born & Bülbring (1955) that adenosine triphosphate levels were not diminished in taenia coli during the excitatory phase of the action of DNP but were depressed about 50 % during its inhibitory phase. However, azide altered mechanical and electrical properties of taenia coli in a manner similar to that of DNP, but did not affect active transport in the uterus. In view of this fact and of the occurrence of numerous differences in the electrical activity of uterine and intestinal smooth muscles (Daniel & Singh, 1958; Singh & Daniel, 1960; Daniel, Honour & Bogoch, 1960; Daniel, 1960) these results may not be transferable to uterine muscle.

SUMMARY

1. Segments of tissue of cat and rabbit uterine muscle were depleted of potassium and enriched with sodium during exposure to cold (4° C) in a potassium-free Krebs–Ringer solution. During recovery from potassium depletion various metabolic inhibitors were employed to study their effects on active extrusion of sodium and reaccumulation of potassium.

2. Anaerobiosis and most inhibitors of oxidations (cyanide, azide, antimycin and malonate) had relatively little effect on active ion transport. Iodoacetate and fluoride, inhibitors of glycolysis, inhibited active transport. However, 2,4-dinitriphenol, pentobarbital and ethanol as well as ouabain, an inhibitor of the ion-carrier system in the membrane in other cells, were also effective inhibitors of active transport. Similar results were obtained with segments of non-pregnant rabbit uterus or those from pregnant cat uterus.

3. Cocaine had no effect on ionic concentration or active transport in uninhibited uterine muscle. It did not prevent the inhibitory action of iodoacetate on potassium reaccumulation but significantly restored the

depressed potassium reaccumulation after DNP, suggesting that DNP may have altered the resting permeability to potassium. Calculations were carried out which suggest that DNP also uncoupled sodium extrusion from potassium uptake.

4. These results are discussed and the suggestion made that active transport in uterine muscle depends in a large part on energy from aerobic glycolyses.

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ANTIHISTAMINE ACTIVITY OF EXTRACTS PREPARED FROM BUFFY-COAT LAYER OF HORSE BLOOD AND FROM OAK GALL

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It has been shown that extracts prepared from leucocytes (Kovacs, 1950; Kovacs & Juhasz, 1951, 1952) and from oak gall (Kovacs & Szabadi, 1950) injected into a guinea-pig, protect the animal against a lethal histamine aerosol. The present experiments confirm these results. In addition, it was found that the injection of extracts of the buffy-coat layer of horse blood reduced the sensitivity of the guinea-pig ileum to histamine. Further, in a guinea-pig protected by oak-gall extract, the histamine aerosol produced characteristic behavioural changes which are attributed to a central action of histamine. The protective action of oak-gall extract against the histamine aerosol was recorded in unanaesthetized and in anaesthetized animals. Some of these results have been reported to the Physiological Society (Feldberg & Kovacs, 1959).

METHODS

Testing the sensitivity of the guinea-pig ileum to histamine

In order to test the sensitivity of the guinea-pig ileum to histamine the following standard procedure was adopted. Guinea-pigs weighing 200–300 g were killed by a blow on the head; immediately afterwards a piece of ileum near the caecum was removed and a piece 7–8 cm long was suspended in a 15 ml. bath containing magnesium-free Tyrode solution at 34° C, through which a mixture of 95% O_2 + 5% CO_2 was bubbled. The contractions of the preparation were recorded on a smoked drum by a frontal-writing lever, using the same lever and the same magnification, which was 1:2, throughout. Immediately the preparation was set up, 0·08 μg histamine (base) was added to the bath for 20 sec, and the size of the contraction was recorded. The administration of 0·08 μg histamine was repeated at least ten times at 2 min intervals so that the ileum developed to the full the response of which it is capable at this dose. If the tenth administration caused no or only a small contraction, larger doses of histamine were given.

Testing for bronchoconstriction produced by a histamine aerosol

Two methods were used to study in guinea-pigs weighing 200–300 g the protective effect of oak-gall extracts on the bronchospasm elicited by a histamine aerosol.

In the one method an unanaesthetized guinea-pig was put into a chamber through which a histamine aerosol was passed and the behaviour of the animal was observed through the

glass walls. The chamber was 15 cm wide, 37 cm long and 27 cm high. A 0·6 or 1% histamine diphosphate solution was passed through the chamber as an aerosol with a Collison inhaler at a pressure of 1/4 atmosphere from a compressed air cylinder.

In the other method a guinea-pig was anaesthetized by subcutaneous injection of a 25% solution of urethane, 6 ml./kg. One to two hours later, when the animal was deeply anaesthetized, it was tied on its back in a specially constructed chamber and the respiratory movements of the chest and abdomen were recorded on a smoked drum, as illustrated in the diagram Fig. 1. The movements of the chest and abdomen are transmitted to a lever via a hinged flap made of Perspex. An upward movement of the flap due to an inspiration causes a downward stroke of the lever. A little spring is attached to the free end of the flap so as to reduce the large sudden excursions which might result, during broncho-constriction, from the intense inspiratory efforts with their wide-spread muscular contractions. A Perspex box (22 cm wide, 35 cm long and 10 cm high) was placed over the anaesthetized

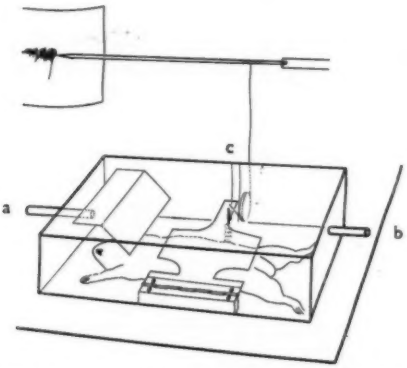

Fig. 1. Diagram of the chamber used to study the effect of a histamine aerosol on the respiratory movements in anaesthetized guinea-pigs. (*a*) Inlet tube for the aerosol. (*b*) Escape tube. (*c*) Narrow slit for the thread connecting the Perspex flap with the lever.

animal and a 1% histamine diphosphate aerosol was passed at a pressure of 1/4 atmosphere from a Collison inhaler through the opening (*a*) and allowed to escape through the opening (*b*) and the narrow slit (*c*).

Preparation of extracts from the buffy coat of horse blood

Crude extracts. Horses which had been immunized, whose plasma was to be used for other purposes, were bled directly from the jugular vein into Winchester bottles containing 10% potassium oxalate (16·5 ml./l. blood). The blood corpuscles were allowed to settle and the plasma was removed.

The corpuscle sediment was kept in the cold for 24 hr–2 weeks before the white layer on top was sucked off and used for preparing the extract. From a batch of 150–200 l. blood about 0·5–1·5 l. of whitish red mush was obtained. The mush was centrifuged and the white layer was sucked off. This procedure was repeated and 300–500 ml. of mush consisting mainly of white cells was obtained.

The mush was frozen during the night at $-5°$ C and allowed to thaw the following morning and then ground for 20–25 min in a mortar with quartz sand acidified with sufficient N-HCl

to give a pH of between 5 and 5·5. By allowing the mixture to stand for a few minutes the mush could be decanted from the sand into a large flask. The sand was repeatedly washed with distilled water and the washings were added to the flask, the final volume being 3–4 l. This mixture was neutralized with N-NaOH to a pH of between 6·9 and 7, and allowed to stand for 10–15 min, during which time a sediment formed which, when centrifuged off, was the crude extract used for injection into guinea-pigs. One millilitre of crude extract corresponded roughly to 2 ml. of the frozen mush or to nearly 1 l. of horse blood.

Ether–chloroform extract. The crude extract was acidified immediately after centrifugation with N-HCl to a pH of 2 and frozen at −5° C. In this condition it could be kept for several weeks apparently without deterioration. For the ether–chloroform extraction the crude extract was thawed, then neutralized with N-NaOH to a pH of 6·8–7·2, and extracted twice with 2–3 times the volume of ether and then twice with 2–3 times the volume of chloroform. The ether and chloroform solutions were pooled and concentrated to dryness on a water-bath between 30 and 40° C. The dry material was kept under nitrogen at −70° C in a small box of CO_2 snow. The yield of dry material from a bath of 150–250 ml. crude extract was 0·5–1·5 g. Before being used for injection the dry material was dissolved in a small quantity of ether, then isopropylmyristate 2 ml. was added to the solution and the ether removed *in vacuo*; the active material remained dissolved in the isopropylmyristate.

Aluminium oxide column chromatography. The ether–chloroform extract was purified by dissolving the dry material in petrol ether and chromatographing it on an aluminium oxide column. The petrol ether had a boiling point of 30–40° C. About 10–12 ml. was used for each gram of dry material and the column was made up of 45–55 g of aluminium oxide suspended in the petrol ether. The height of the column was 12–13 cm and its diameter was 2·5 cm. The procedure of elution which was carried out under nitrogen was as follows:

1. 100 ml. petrol ether (30–40° C).
2. 100 ml. petrol ether (40–60° C).
3. 50 ml. petrol ether (40–60° C) + 50 ml. petrol ether (60–80°).
4. 100 ml. petrol ether (60–80° C).
5. 95 ml. petrol ether (60–80° C) + 5 ml. chloroform.
6. 90 ml. petrol ether (60–80° C) + 10 ml. chloroform.
7. 80 ml. petrol ether (60–80° C) + 20 ml. chloroform.
8. 70 ml. petrol ether (60–80 °C) + 30 ml. chloroform.
9. 60 ml. petrol ether (60–80° C) + 40 ml. chloroform.
10. 50 ml. chloroform + 50 ml. ether.
11. 250 ml. ether.

Each of the eleven fractions was dried separately *in vacuo*, taken up with 5 ml. ether and divided into two portions of 2 and 3 ml. which were then dried *in vacuo* and kept frozen under nitrogen at −70° C until tested. The 2 ml. portion was used to find out if it had a protective effect against a lethal histamine aerosol in an unanaesthetized guinea-pig; and if so the other 3 ml. was tested for its effect on the sensitivity of the ileum to histamine. Immediately before the injections the portions were dissolved in 0·5–2 ml. isopropylmyristate and injected in this form. Control injections of similar amounts of isopropylmyristate were ineffective.

Preparation of alcoholic extract from oak gall

Hungarian oak galls, the tumours produced by the larvae of *Cynips quercus calicis*, were used. The galls had been stored at −5° C for 2 years. For each experiment 4–6 galls, weighing 18–23 g, were ground in a special plant mill; the powder was extracted with 200 ml. ethanol at 65° C for 5 min, cooled, filtered and re-extracted in the same way with 100 ml. ethanol. The combined filtrate, a brownish alcoholic solution which contains the active principle, amounted to between 210 and 240 ml. Two samples, each of 4–6 ml., were immediately evaporated to dryness *in vacuo*. One sample served to determine the dry weight, which was

between 10 and 15 mg/ml. filtrate, so that the alcohol filtrate from 20 g of the original powder contained between 2 and 3 g dry material. The other sample was taken up in 3–5 ml. saline solution, of which usually half was used for injection into a guinea-pig. The amount of dry weight injected was thus between 20 and 45 mg, usually between 30 and 35 mg.

RESULTS

Effect of injections into guinea-pigs of extracts of the buffy coat of horse blood on the histamine sensitivity of the ileum preparation

In preliminary experiments it was found that extracts of the buffy coat of horse blood added to the guinea-pig ileum preparation suspended in magnesium-free Tyrode solution had a weak and irregular depressant effect on histamine contractions. On the other hand, the histamine sensitivity of ileum preparations obtained from guinea-pigs injected with these extracts was lower than that of preparations obtained from untreated control guinea-pigs.

In untreated control guinea-pigs, the sensitivity of the ileum preparation to histamine tested under the standard conditions described showed surprisingly small individual variations. The range of variation is illustrated in Fig. 2, which shows the development of the histamine responses in a sensitive, an insensitive and an intermediate preparation obtained from different guinea-pigs.

Experiment *A* illustrates the development of the responses in a sensitive preparation. The first administration of 0·08 μg histamine produced a strong contraction, and with each subsequent administration the contractions increased until a maximum was reached after the twelfth administration. At this stage half the dose of histamine (0·04 μg) was sufficient to produce a strong contraction.

On the other hand, in experiment *C*, the first two doses of 0·08 μg histamine were ineffective, but even in this insensitive preparation a relatively strong contraction developed after ten administrations of 0·08μg histamine, and maximal sensitivity was reached after about twenty administrations; and at this stage 0·04 μg histamine also produced a contraction. This preparation illustrates the development of histamine responses as it occurs in insensitive preparations encountered in control guinea-pigs. The development of the responses in a preparation of intermediate sensitivity is illustrated in experiment *B*.

Crude extract. When guinea-pigs were killed a few hours after an intraperitoneal injection of 5–7 ml. of crude extract, and the abdomen was opened, it could be seen that the extract was not fully absorbed. The appearance of the intestine varied; sometimes it had a normal appearance, sometimes it had a greyish colour and was fully relaxed, but the appearance bore no relation to the sensitivity of the ileum preparation to histamine.

When an intraperitoneal injection of the crude extract had been effective in protecting the unanaesthetized guinea-pig against a lethal histamine aerosol, it had also resulted in a reduced sensitivity of the ileum preparation to histamine. This result was obtained with extracts prepared from ten batches of buffy-coat layer. With five the reduction in sensitivity of the ileum preparation to histamine was 1000 times or more, with the other five it was between 2 and 20 times. There was also a reduction in sensitivity to acetylcholine but to a lesser degree. Ileum preparations of untreated guinea-pigs responded usually to 0·04 μg acetylcholine with a contraction

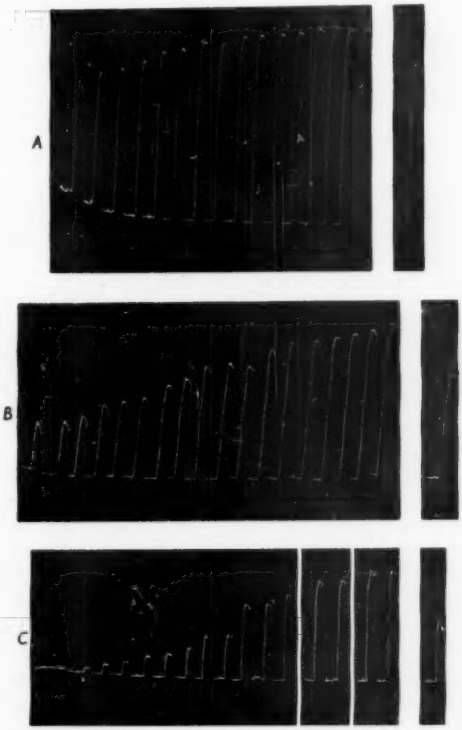

Fig. 2. Development of histamine responses in three ileum preparations from different untreated guinea-pigs. Preparations suspended in 15 ml. magnesium-free Tyrode solution. All contractions except the last ones were in response to 0·08 μg, the last ones to 0·04 μg, histamine. *A*, a sensitive preparation; *B*, a preparation of average sensitivity; and *C*, a relatively insensitive preparation, which did not respond to the first two administrations of 0·08 μg histamine, which are indicated by the white dots. Between each of the two gaps after the first and second blocks at *C* two contractions have been omitted.

equal to that produced by 0·08 μg histamine, the ratio acetylcholine: histamine being about 1:2. In preparations of treated animals, in which the sensitivity to histamine became greatly, and that to acetylcholine slightly reduced, the ratio could become 1:100 or even 1:1000.

The result with a particularly effective extract is illustrated in Fig. 3*A*. The guinea-pig had been injected intraperitoneally with 7 ml. extract and was killed 7 hr later. The ileum preparation from this guinea-pig did not

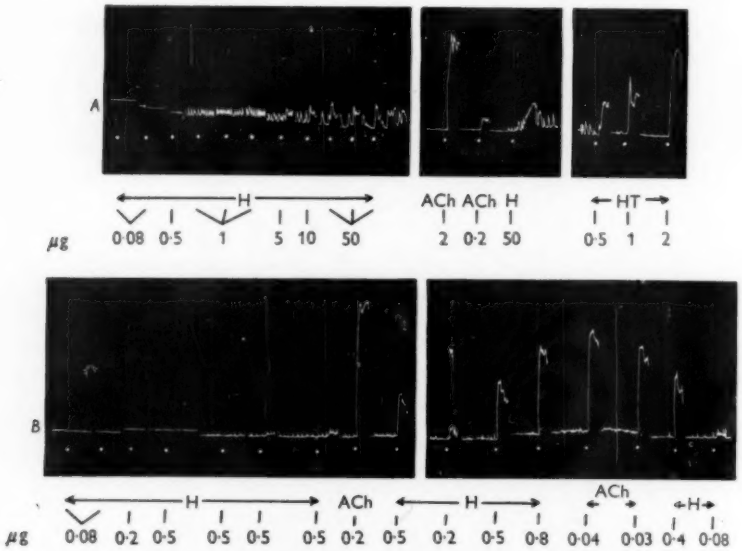

Fig. 3. Development of histamine (H), acetylcholine (ACh) and 5-hydroxytryptamine (HT) responses in ileum preparation, suspended in 15 ml. magnesium-free tyrode solution, from guinea-pigs killed 7 hr after (*A*) an intraperitoneal and (*B*) a subcutaneous injection of 7 ml. crude extract from buffy coat of horse blood: numbers indicate doses. For details see text.

respond to 0·08 μg histamine. In Fig. 3*A* only the last two of ten administrations of 0·08 μg are shown. When the dose of histamine was increased to 50 μg still no contraction ensued. This experiment illustrates also that the sensitivity of the ileum preparation to acetylcholine was reduced. It responded to 0·2 μg acetylcholine with a small, and to 2 μg with a strong, contraction, at a time when it was nearly insensitive to 50 μg histamine. The sensitivity to 5-hydroxytryptamine (5-HT) was reduced as well, but the preparation responded to 0·5 μg 5-HT. Since preparations from untreated guinea-pigs are less sensitive to 5-HT than to histamine, the reduction in 5-HT sensitivity was less than that of acetylcholine.

When the same extract had been injected subcutaneously the reduction in sensitivity of the ileum preparation was less pronounced than after intraperitoneal injection, as is shown in Fig. 3*B*. This ileum preparation again was insensitive to 0·08 μg histamine. The record begins with the last two of ten administrations. At this time the preparation was also insensitive to 0·2 and 0·5 μg histamine, but later it responded to 0·2 with a slight, and to 0·8 μg histamine with a relatively strong response. The sensitivity to acetylcholine was little if at all reduced, since the preparation responded to 0·03 μg acetylcholine with a contraction equal to that produced by 0·8 μg histamine.

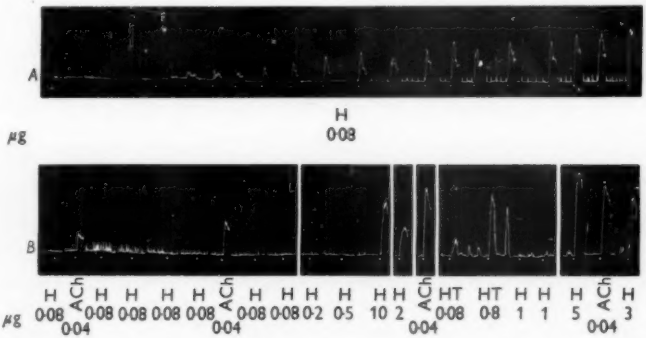

Fig. 4. Development of histamine (H), acetylcholine (ACh) and 5-hydroxytryptamine (HT) responses in ileum preparations, suspended in 15 ml. magnesium-free tyrode solution, from guinea-pigs killed 6 hr after a subcutaneous injection (*A*) of 2·5 ml. and (*B*) of 5 ml. crude extract from buffy coat of horse blood: numbers indicate doses. For details see text.

The degree of reduction in sensitivity depends on the amount of crude extract injected intraperitoneally or subcutaneously. This is shown for subcutaneous injections in Fig. 4. The extract was from a different batch of horse blood from that used in experiment of Fig. 3. The preparation from a guinea-pig injected with 2·5 ml. extract was only a little less sensitive to histamine than an insensitive preparation from a control guinea-pig, since with repeated administration of 0·08 μg histamine contractions were obtained (Fig. 4*A*). On the other hand, a strong reduction in sensitivity was obtained in the preparation from a guinea-pig injected with double the amount of extract (Fig. 4*B*). The sensitivity to acetylcholine was apparently unchanged. When full sensitivity had developed, 0·04 μg acetylcholine gave a contraction stronger than that of 3, and weaker than that of 5 μg histamine, so that the ratio acetylcholine:histamine was about 1:100. There was apparently no decrease in sensitivity to 5-HT,

since the preparation responded to 0·8 μg 5-HT with a strong, and to 0·08 μg with a weak, contraction.

Ether-chloroform extract. Not all ether–chloroform extracts prepared from active crude extract were effective, on intraperitoneal injection, in giving protection against a lethal histamine aerosol or in reducing the sensitivity of the ileum preparation to histamine. Further, when an active ether–chloroform extract was kept, even at −70° C, for a few days, it usually became less active. This instability of the active principle in the extract may well explain why some extracts were inactive from the beginning.

Some ileum preparations from guinea-pigs to which about 60 mg of ether–chloroform extract had been injected 20 hr before the animals were killed contracted only to 20 or 50 μg histamine. The sensitivity to acetylcholine was also reduced, but to a lesser extent. A dose of 0·2 μg acetylcholine was always effective and in one experiment the strong reduction

Fig. 5. Development of histamine responses in an ileum preparation, suspended in 15 ml. magnesium-free tyrode solution, from a guinea-pig killed 20 hr after an intraperitoneal injection of 1 mg dry weight of fraction 8 obtained on elution of an ether–chloroform extract from buffy coat of horse blood after chromatography on an alumihium oxide column: numbers indicate doses. For details see text.

in sensitivity to histamine occurred without an apparent change in sensitivity to acetylcholine. A dose of 20 μg histamine produced a contraction equal to that of 0·02 μg acetylcholine, so that the acetylcholine:histamine ratio was 1:1000.

Aluminium oxide column chromatography. Of the 11 fractions obtained nos. 2, 3, 4, 5, 6 and 11 did not show a protective effect against a lethal histamine aerosol when injected intraperitoneally into a guinea-pig, but with one or more of the fractions 1, 8, 9 and 10 protection was obtained in six experiments. Yet in only two of these did extract (1 mg) produce also a definite reduction in sensitivity of the ileum preparation. One of these experiments is illustrated in Fig. 5. The ileum responded to 5 μg with a just perceptible contraction, and the responses to 10 and 50 μg were weaker than those obtained with 0·08 μg in control preparations.

Since a definite reduction in sensitivity was obtained in two experiments only, the failure to obtain this effect in the other four experiments was thought to have been due to the fact that the amount of extract injected with each fraction was too small. Therefore, in one experiment three

effective fractions from three different batches of horse-blood buffy coat were pooled. The effective fractions from the first and second batches were stored at $-70°$ C until the third effective fraction was obtained. The time of storage was a fortnight for the fraction of the first, and a week for that of the second batch. The dry weight of the three fractions was 2·5 mg. When this was injected intraperitoneally, the ileum preparation of the guinea-pig killed 24 hr later did not respond to 100 μg histamine.

Effect of a histamine aerosol on guinea-pigs injected with oak-gall extracts

Unanaesthetized guinea-pigs. The observations were confirmed which had been made by Kovacs & Szabadi (1950) on the protective action of an intraperitoneal injection of an alcoholic extract obtained from oak gall against the lethal effect of a histamine aerosol in unanaesthetized guinea-pigs. The typical effect of a lethal histamine aerosol on an untreated normal guinea-pig is as follows. A few seconds after the beginning of the dispersion of a 0·6–1 % histamine aerosol into the chamber which houses the guinea-pig, its respiration accelerates. A little later the respiration becomes laboured with apparently unsuccessful inspiratory efforts and there is intense gasping. The animal quickly becomes cyanotic and has a frightened aspect. Next it becomes unsteady, swaying from side to side, and rears up suddenly when making an effort to get air into its lungs. It then falls on its side or back, and within a few seconds lethal general convulsions develop which last a few seconds. After cessation of the convulsions there is often urination and sometimes superficial movements of the thorax and abdomen occur and continue for a short time before the animal dies. An untreated guinea-pig usually does not survive the aerosol for more than a few minutes.

A guinea-pig protected aginst a lethal histamine aerosol by an intraperitoneal injection of an alcoholic extract of oak gall (dry weight 20–45 mg) survives the aerosol for over 20 min and sometimes for over 60 min, and when it dies death is not from bronchospasm but apparently from a central action of histamine. The effects of 43 freshly prepared extracts were examined. On intraperitoneal injection a strong protection, i.e. a survival time of at least 20 min during exposure to the histamine aerosol, was obtained with 26 extracts; a less pronounced protection with a survival time of about 10 min was obtained with 10 extracts, and with 7 extracts there were no definite signs of protection, i.e. no prolongation of survival time.

During the survival time the protected animal shows characteristic changes in behaviour. The typical effect of a 0·6–1 % histamine aerosol in a fully protected animal is as follows. Acceleration of respiration, as observed in the unprotected animal, usually occurs and continues for some

time, but there are no signs of unsuccessful inspiratory efforts. At the time when an unprotected animal would be entering the stage of lethal convulsions, the protected animal shows no signs of any further histamine effects. Later, after about 3–5 min, the respiration becomes slower and deeper, and is interrupted from time to time by a deep inspiration, without apparent bronchoconstriction, followed by a period of apnoea. Without rearing or convulsion the animal may fall on its side or take up a crouching position with its head flexed. The deep inspirations followed by short periods of apnoea become more frequent and dominate the pattern of respiration. When at this stage the animal is taken out of the chamber it can be put on its back and makes little or no effort to right itself. And when placed on its belly, the hind legs are not drawn under the body but remain sticking out in a half-flexed position resembling that usually taken up by frogs. This is the result of definite weakness of the legs, particularly the hind legs, which can be flexed and extended without much resistance. The tendon reflexes are present and when the hind paw is pinched the animal withdraws its leg and tries to move away, but the reaction is delayed and more sluggish than in a normal animal. The appearance is that of an animal slightly anaesthetized. When the animals are kept in the aerosol they do not die from bronchospasm, but the respiration becomes slower and deeper, and finally ceases after a few superficial ineffective respiratory movements.

The same difference between the unprotected and the protected un-anaesthetized animal was observed when a lethal dose (histamine base 0·4 mg/kg) was injected into the vein on the back of the thigh. The unprotected animal died within a few minutes, whereas the protected animal showed the characteristic central effects of histamine, a condition of weakness or partial paralysis of the hind legs, light anaesthesia and slow respiration. The protected animal tolerated 1 mg/kg of histamine or more.

Anaesthetized guinea-pigs. The differences in the respiratory response to a histamine aerosol is also observed in guinea-pigs anaesthetized with urethane. Figure 6 shows the typical lethal effect of a 1% histamine aerosol on respiration in three anaesthetized untreated guinea-pigs. The two animals from which the upper records were taken died within 3 min from intense bronchospasm, the last gasp being shown in both records. The sequence of respiratory changes was usually as follows. Within 20–30 sec of the beginning of exposure to the histamine aerosol the animal would take a deep breath and then there would be a short period of superficial polypnoea indicated in the records by a gradual upward movement of the lever, without showing the individual respiratory movements. The respiratory movements then became deeper and there followed a period of laboured respiration with inspiratory dyspnoea. Each forced inspiration gave a

downward stroke on the record; some of the inspiratory efforts were so intense that they involved almost the whole body musculature. In the records they produced the very large downward strokes. Very quickly the forced respiratory movements became less frequent, periods of apnoea occurred which lengthened; from time to time they were interrupted by intense inspiratory efforts involving the whole body musculature. Sometimes, after respiration had stopped for 30 sec or more, a few ineffective respiratory movements reappeared; this happened also after the aerosol

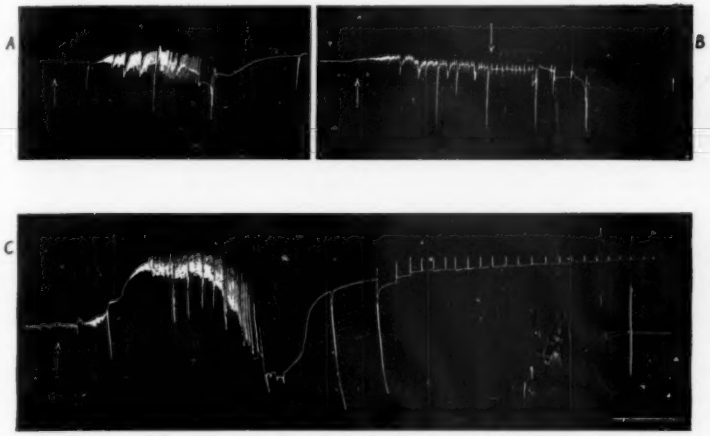

Fig. 6. Records of respiration in three guinea-pigs anaesthetized with urethane; lethal effect of a 1 % histamine aerosol. Beginning of exposure to aerosol indicated by arrow (↑). In guinea-pig *B*, the aerosol was discontinued after 75 sec at second arrow (↓). Time marker, 30 sec. For details see text.

had been discontinued, but they did not bring air into the lung and did not affect the lethal outcome. In the experiment of Fig. 6*C* there was a period of about 3 min of ineffective, gradually decreasing respiratory movements which occurred every 7–8 sec.

Between 30 and 35 mg dry weight of an alcoholic extract of oak gall was required to produce the protection on intraperitoneal injection against the lethal histamine aerosol. Protection occurred within 1 hr and lasted for 24 hr, or sometimes as long as 4 days. In the experiment of Fig. 7 the animal was tested 4 hr and again 4 days after the intraperitoneal injection of the oak-gall extract. Four hours after the injection no signs of bronchoconstriction were produced by the histamine aerosol, but deep inspirations interrupted the normal breathing and became more frequent as the aerosol continued. As shown in the later records (20 and 25 min) the respiration between the deep inspirations became progressively slower and finally

(after 30 min) there was no longer a distinction between deep inspiration and normal respiration, which had become slow, about one respiration every 3 sec. When retested 4 days later (lower record Fig. 7 *B*) the effect of the histamine aerosol had scarcely changed; however, when retested 5 days later (not shown in the figure) the histamine aerosol caused intense bronchoconstriction and the animal died within 5 min.

The experiment of Fig. 8 illustrates in another animal the protection attained 1 hr after an intraperitoneal injection of the oak gall extract. In this experiment the histamine aerosol was discontinued after 30 min (at the arrow in *e*). Respiration soon quickened and the deep inspirations became less frequent and ceased within 7 min.

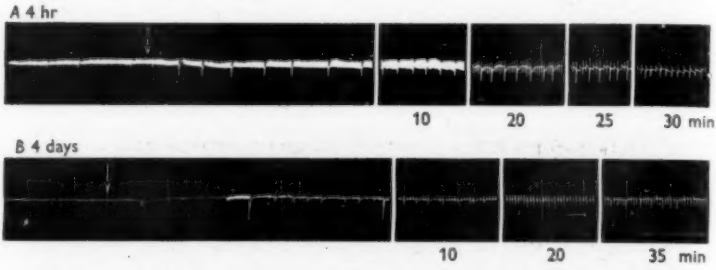

Fig. 7. Records of respiration from a guinea-pig under urethane anaesthesia (*A*) 4 hr and (*B*) 4 days after an intraperitoneal injection of an alcoholic extract of oak gall. At the arrows (↓) beginning of exposure to 1 % histamine aerosol, which was continued until the end of the records; the figures below the records show the time in minutes after the beginning of exposure to the histamine aerosol. Time marker, 30 sec. For details see text.

When only about half the amount of alcoholic extract, corresponding to 15 mg dry weight, was injected intraperitoneally, only slight protection was attained.

The alcoholic extract of oak gall was also effective when injected subcutaneously, but the effect was not so pronounced as after intraperitoneal injection. A typical experiment is illustrated in Fig. 9 in which the histamine aerosol was tested 3½ hr after a subcutaneous injection of an alcoholic extract of oak gall (dry material 35 mg). The extract was from the same batch as that tested in experiment Fig. 8. The animal withstood the histamine aerosol which was continued for 30 min, but at the beginning there was one strong inspiratory effort, as indicated by the large downward stroke which indicated some bronchoconstriction. When the animal was retested 24 hr later, the protection against the histamine aerosol had practically disappeared.

The protective action of the alcoholic extract of oak gall does not result

from the tannic acid present in the extract, because tannic acid injected intraperitoneally into a guinea-pig did not protect the animal, whereas an alcoholic extract of oak gall, from which the tannic acid had been removed by lead hydroxide precipitation, produced protection. The experiment of Fig. 10 shows the protection against a histamine aerosol tested 5 hr after an intraperitoneal injection of about 10 mg tannin-free dry material.

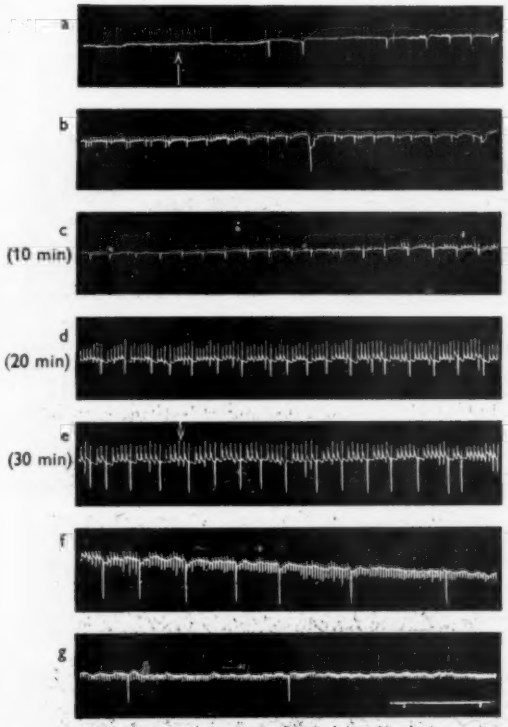

Fig. 8. Records of respiration from a guinea-pig under urethane anaesthesia 1 hr after an intraperitoneal injection of an alcoholic extract of oak gall (dry weight 35 mg). At the arrow (↑) at *a*, beginning of a 30 min exposure to 1 % histamine aerosol which was discontinued at the arrow (↓) at *e*. Tracing *a* and *b*, continuous record; *c*, 10 min, *d* 20 min and *e* 30 min after beginning of histamine aerosol; tracing *e*, *f* and *g*, continuous. Time marker, 30 sec. For details see text.

DISCUSSION

The finding that extracts of the buffy coat of horse blood injected into a guinea-pig selectively lower the sensitivity to histamine, not only of the smooth muscles of the bronchi but also of the intestine, suggests that the

active principle of the extracts affects smooth muscle in general. The present experiments do not deal with the problem of the origin of the active principle in the buffy-coat layer. When Kovacs (1950; Kovacs & Juhasz, 1951, 1952) examined the antihistamine effect of such extracts on the bronchi in guinea-pigs *in vivo*, he found a definite correlation between potency and eosinophil count of the extracts, and suggested that the active principle was derived from the eosinophils. This suggestion is supported by the observation that extracts prepared from isolated eosinophil granules, obtained by digestion of leucocytes with trypsin, exert an antihistamine effect on the isolated guinea-pig ileum (Vercauteren & Peeters, 1952; Vercauteren 1953). Recently, Archer, (1956, 1959)

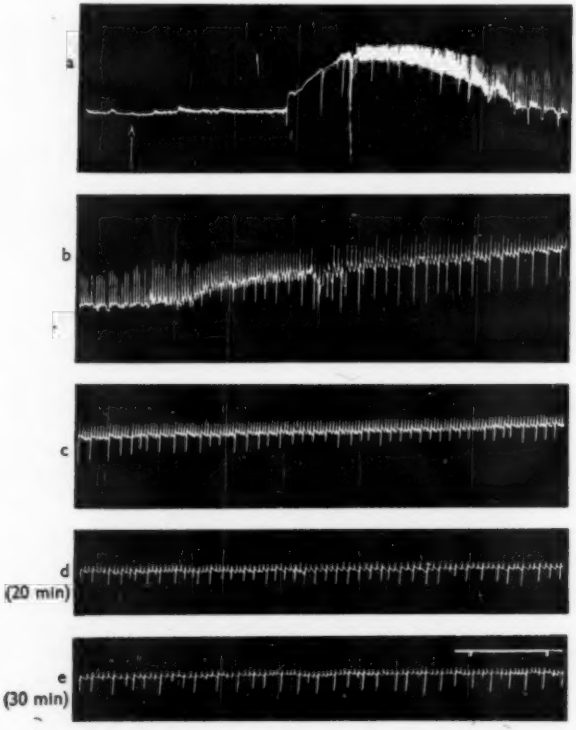

Fig. 9. Record of respiration from a guinea-pig under urethane anaesthesia, 3½ hr after a subcutaneous injection of an alcoholic extract of oak gall (dry weight 35 mg). At the arrow (↑), beginning of exposure to a 1% histamine aerosol which was continued until the end of the record. Tracings *a*, *b* and *c*, continuous record; *d* 20 min and *e* 30 min after the beginning of the histamine aerosol. Time marker, 30 sec. For details see text.

attributed antihistaminic properties to the eosinophils. According to his view the eosinophil leucocytes have a chemotactic affinity to histamine, since he found that injections of histamine into the bone marrow or into the skin of horses produce a local accumulation of eosinophils. Their antihistaminic property was based on the finding that the local oedema normally produced by an intradermal injection of histamine was reduced or abolished in the eosinophil-rich areas. If the active principle of our extracts prepared from the buffy-coat layer were derived from the eosinophils it would not be surprising that the amount of extract required to

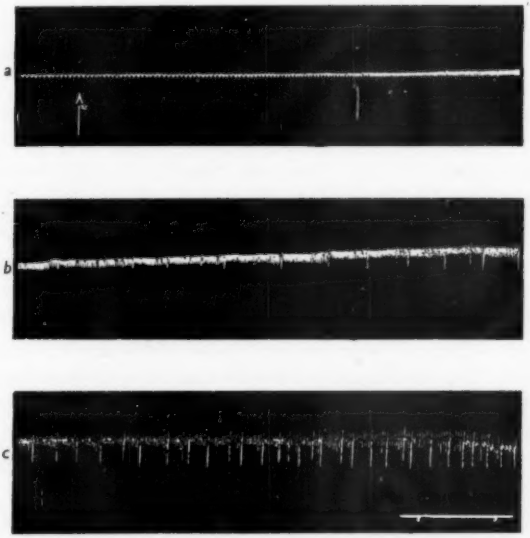

Fig. 10. Record of respiration from a guinea-pig under urethane anaesthesia, 5 hr after an intraperitoneal injection of tannic-acid-free extract of oak gall (dry weight 10 mg). At the arrow (↑) beginning of exposure to 1 % histamine aerosol, which was continued to the end of the record. Tracings *a*, *b* and *c*, continuous record. Time marker, 30 sec. For details see text.

produce the antihistaminic effect corresponds to a relatively large amount of the buffy coat, since it is known that on standing and sedimentation of blood the eosinophils have a tendency to pass into the layer of red cells (R. K. Archer, personal communication).

The fact that the extract of the buffy coat layer had no, or only a weak, antihistaminic effect *in vitro* when added to the bath in which the ileum preparation was suspended can be explained in either way. The active principle may be water-insoluble and therefore not effective when added to the bath fluid, or the effect may not be a direct one on the smooth

muscles, as that of the synthetic antihistamine, but may develop only *in vivo* by an as yet undefined mechanism. It is natural to think in this connexion of the protective action which cortisone and adrenocorticotrophic hormone exert on allergic and inflammatory reactions.

One of the striking features of the antihistaminic action of the alcoholic extracts of oak gall was its long duration. The variability in the effectiveness of our different batches of oak-gall extracts is in agreement with previous observations (Kovacs, Kovacs, Szabady & Varsanyi, 1952). Since the active principle in oak galls seems to be inactivated by oxygen, such variability is more likely to be found when, as in the present experiment, old stored galls are used for extraction. This is because in spring the wasps escape from the galls, leaving fine holes in the skin through which air can enter.

When a guinea-pig was protected against a lethal histamine aerosol by an injection of oak-gall extract the histamine aerosol was not found to be ineffective, but produced changes in respiration and behaviour which were attributed to central actions of the histamine. The guinea-pigs appeared to be partly paralysed or in a condition of light anaesthesia. In other species histamine is known to produce such effects as a result of its central action (for references see Feldberg & Schilf, 1930).

In the untreated guinea-pigs such effects have hitherto not been observed because of the lethal bronchospasm which supervenes so quickly. By protecting the guinea-pigs against this bronchospasm these central effects are unmasked. This does not necessarily mean that the oak-gall extracts have no protective action against the central effects of histamine, it need only be a sign that the effects of histamine on the central nervous system are more resistant than those on the bronchial muscles.

From the experiments performed so far it is not possible to draw conclusions about the chemical nature of the active substance in oak-gall extracts or about its mechanism of action. Nor is it possible to state whether the active principle is the same as that effective in extracts of the buffy-coat layer of horse blood.

SUMMARY

1. In guinea-pigs protected against a lethal histamine aerosol by an intraperitoneal injection of extract prepared from the buffy-coat layer of horse blood, the ileum, when suspended in a bath of tyrode solution, was found to be two to a thousand times less sensitive to histamine than a preparation obtained from an untreated animal. The sensitivity to acetylcholine was also reduced, but to a lesser degree.

2. An intraperitoneal injection of the extract was more effective than a subcutaneous one.

3. The reduced sensitivity of the ileum preparation was observed a few hours after the injection. When a purified extract prepared with Al_2O_3 column chromatograhy was used, the effect lasted for at least 24 hr.

4. Guinea-pigs were protected against a lethal histamine aerosol by intraperitoneal or subcutaneous injections of an alcoholic extract prepared from Hungarian oak gall.

5. In untreated guinea-pigs a 0·6–1% histamine aerosol produces lethal bronchospasm, and the animals usually die within a few minutes. Protected animals withstood the histamine aerosol for 20 min or longer, and death when it occurred was apparently due to central respiratory failure.

6. These effects were obtained in unanaesthetized guinea-pigs and in guinea-pigs anaesthetized with urethane, in which the respiratory movements during the histamine aerosol were recorded on a smoked drum by transmission of the movements of the chest and abdomen to a lever through a hinged flap made of Perspex.

7. In unanaesthetized guinea-pigs protected by an injection of oak-gall extract the histamine aerosol produced not only changes in respiration but also in behaviour. The animals appeared to be partially paralysed or in a condition of light anaesthesia. This is probably a central effect of histamine, known to occur in other species, and unmasked in the guinea-pig because the lethal bronchospasm was prevented.

8. The protective effect of oak-gall extract developed within 1 hr after the injection; it lasted for 24 hr and sometimes as long as 4 days.

9. The protective effect of the oak-gall extract was not due to tannic acid. An injection of tannic acid was ineffective, but an extract from which the tannin had been removed by lead hydroxide precipitation produced the effect.

We wish to thank the Distillers Company (Biochemicals) Ltd., London, for their generous gift of isopropylmyristate. One of us (B.A.K.) would like to make grateful acknowledgement to the Wellcome trust for a personal grant, during the tenure of which the greater part of this work was done.

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THE PROPERTIES OF SINGLE-UNIT DISCHARGES IN THE OPTIC LOBE OF THE LOCUST

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In the course of previous work in which micro-electrodes have been used to record nervous activity in the locust optic lobe (Burtt & Catton, 1956, 1959*a*), from time to time the activity of single units has been observed, although in general the responses have been multiple in nature. The present paper deals with the collected data from experiments on thirty-one single units on which extended observations could be performed. A preliminary report has already appeared (Burtt & Catton, 1959*b*).

METHODS

The preparation. Adult female specimens of the migratory locust, *Locusta migratoria migratorioides* R & F, were used in all experiments. The insect was immobilized by dividing the nerve cord in the cervical region, and was firmly fixed on a cork platform. The head was secured in a Plasticine mould, and a small pilot hole was made centrally in the cornea to permit entry of the micro-electrode. An indifferent electrode was inserted through a hole in the head. In some experiments the head capsule was opened and the micro-electrode was inserted into the anterior or posterior lateral surface of the optic lobe (Fig. 1).

Stimulation and recording. The types of stimulation used were (*a*) on–off, by switching a lamp placed over the preparation on and off; (*b*) movements of a small illuminated white card to evoke movement responses. The metal micro-electrodes were made by inserting fine silver or platinum wires into drawn-out glass capillaries and sealing off the tip by gentle heating. Standard silver wire 25 μ in diameter was sharpened by electrolytic erosion in dilute nitric acid to a final tip diameter of 5–10 μ. Wollaston wire was found convenient for making platinum electrodes, a short terminal portion of the 10 μ platinum core being exposed by dissolving away the silver sheath in nitric acid, leaving the stouter silver-sheathed portion for soldering to a lead-out wire. The a.c.-coupled amplifier was used with a short time constant in order to attenuate unwanted low-frequency signals such as the illumination potential. The micro-electrode was inserted by means of a micrometer drive reading to 50 μ.

RESULTS

During penetration of the eye and optic lobe by the micro-electrode there was a sudden onset of large spike activity when the electrode tip reached a level corresponding to the outer border of the second synaptic region, about 1·1 mm from the eye surface (Fig. 1). Although in many

experiments the activity of more than one unit was seen in the records, in favourable cases it was possible to identify the spikes from a single cell by virtue of the regularity of their discharge and uniformity of amplitude, and these were usually detected on both the monitor oscillograph and the loudspeaker in the course of the experiment. The single-unit discharges were recordable only over a very narrow range in depth, not exceeding 0·1 mm in any one instance, indicating the high degree of localization to be expected when recording from a single active cell.

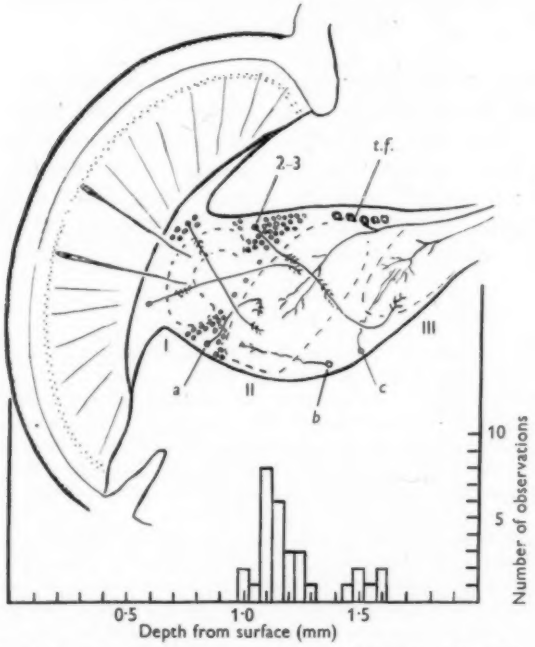

Fig. 1. Frontal section through compound eye and optic lobe of locust, with diagrammatic representation of the three synaptic regions (I, II, III) and some principal fibre connexions. *a*, *b* and 2–3, neurones associated with the second synaptic region; *t.f.*, neurone somata of tract by-passing third region; *c*, neurone associated with the third region. Below is a depth scale, with frequency of observation and depth of single-unit responses.

Of the 31 single units on which extended observations were possible, 25 were found in the depth range 1·0–1·25 mm and 6 in the range 1·5–1·6 mm; the latter did not appear to differ in their properties from the former. One unit giving a very large spike (amplitude about 1 mV) was recorded by the method of lateral approach. It appeared to correspond to

one of a group of large neurones which have been identified anatomically (Fig. 1, *t.f.*), but extended observations were not possible because of the short survival time of the preparation with an open head capsule. In general the behaviour of the single units was characterized by brief high-frequency discharges when the light was switched on or off, although a few units failed to give an off-response. Most units discharged continuously at low frequency in light or darkness, but some (here called *L*-units) were inactive in darkness. Most of the units gave brief discharges in response to movement stimuli, as when a white card was moved rapidly in the visual field. It became clear that at least three types of responding units were present.

Types of single unit response

Those units which gave a steady discharge in the dark at a rate equal to or greater than that observed in steady light, were termed *D*-units. Units which gave a discharge only under illumination of the eye were termed *L*-units. Two units were found which gave pure on-responses.

D-units. These were characterized by giving both on and off peaks of high frequency, by showing a dark-adapted discharge rate either equal to or greater than the light-adapted rate, and by giving responses to movement stimuli. Of the 31 units studied, 24 were of this type. The dark- and light-adapted rates remained constant over long periods. Figures 2 and 3 illustrate an experiment performed on a *D*-unit, which showed a higher dark-adapted than light-adapted rate. Beginning with a period of adaptation to a low intensity (220 lm/m^2) at which the discharge rate was 10/sec, the lamp was quickly moved close to the eye, giving a peak discharge (on-response) of 75/sec. There was a rapid adaptation to the higher intensity (3500 lm/m^2), and within 25 sec the light-adapted rate of 6/sec was reached. Shortly afterwards the light was switched off and the transient off-burst reached a peak of 80/sec, with a very rapid adaptation in about 5 sec to the dark-adapted rate of 20/sec. Figure 4 shows the behaviour of a *D*-unit in which dark- and light-adapted rates were equal. The on and off peak discharge rates were about equal, and adaptation to light and to darkness was initially very rapid, although complete adaptation required about 3 min in each case. Dark discharge rates ranged from 1/sec to 20/sec in different *D*-units, and peak on and off bursts ranged from 40/sec to 150/sec.

L-units. These were a second distinct type of unit, showing no discharge in darkness or below a certain low level of intensity, an on-response, a steady discharge in light, which increased with increasing intensity, and simple cessation of the discharge at 'off'. Five of the units studied were of this type. They were further distinguished from the *D*-units by having a larger spike amplitude and by failing to give responses to movement

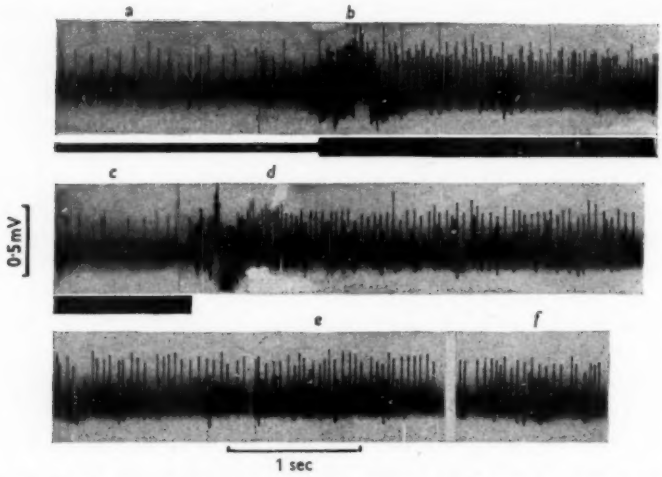

Fig. 2. Oscillogram showing behaviour of a *D*-unit. *a*, adapted rate of discharge in dim light (about 220 lm/m²); *b*, on-response to a sudden increase in intensity by moving of lamp nearer to eye (about 3500 lm/m²); *c*, light-adapted for 30 sec; *d*, off-response; *e*, 30 sec and *f*, 60 sec of dark adaptation. Bar below record indicates illumination of eye and roughly its intensity. Note the base-line disturbances at 'on' and 'off', due to illumination potential.

Fig. 3. Discharge rate of *D*-unit of Fig. 2 plotted against time.

stimuli. The behaviour of a *L*-unit, when subjected to a 3 min period of illumination after a period in darkness, is shown in Fig. 5.

On-unit. Two units were found which gave a response only to 'light on', with a brief following train of spikes which disappeared in a few seconds (Fig. 6). Since these units gave no continuous discharge either in light or in darkness they were regarded as a distinct type of unit.

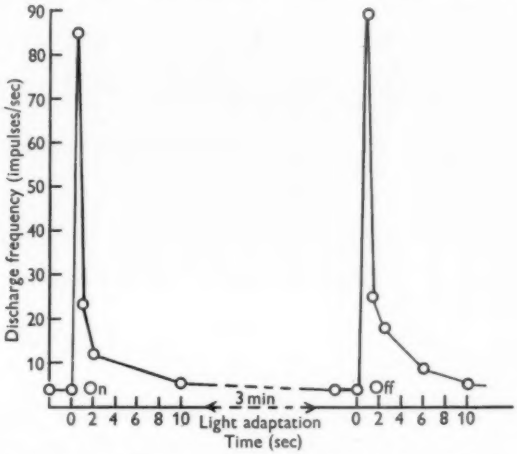

Fig. 4. Behaviour of a *D*-unit in which dark- and light-adapted rates were equal. Record starts in dark-adapted state, followed by a period of 3 min illumination.

Visual field response area of single units

From previous work on the anatomy of the optic lobe and analysis of the synaptic connexions (Burtt & Catton, 1959*a*) it was expected that neurones at the depths at which the single active units were recorded would be of the second or third order removed from the photoreceptors (retinula cells of the ommatidia). It would be reasonable to assume that the single units studied were situated at points of convergence on pathways derived from many ommatidia. If this were so, it should be possible to evoke responses in the *D*-units to movement stimuli extending over a wider angular zone than that associated with a single ommatidium. This latter value was known from previous work (Burtt & Catton, 1954) to be about 20°. It was found that for all the *D*-units tested movement responses could be obtained over the whole visual field of the compound eye, pointing to a high degree of convergence on these units. An extensive overlapping of the post-retinal fibres in their course to the first synaptic region has been clearly seen in the eye of *Calliphora*, not so clearly in *Locusta* (Burtt & Catton, unpublished), in such a way that fibres originating from the same

ommatidium may have widely dispersed connexions in this region. Further complexity of connexions to neurones associated with the second synaptic region may well be introduced by the broad bands of fibres forming the first chiasma, although individual fibre connexions cannot be followed here.

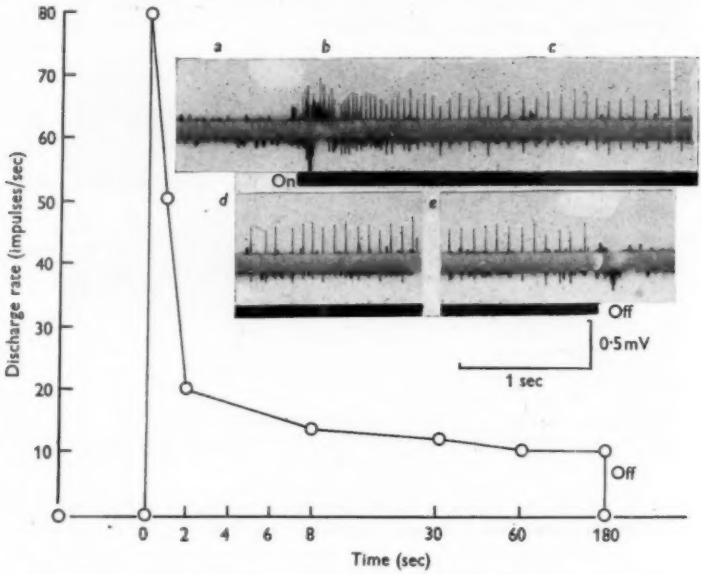

Fig. 5. Behaviour of an *L*-unit. Oscillogram shows *a*, no dark discharge; *b*, on-response (with discharge in other units giving smaller spikes and small on-wave of illumination potential); *c*, sustained discharge during illumination; *d*, 60 sec later; *e*, 3 min later. Bar below record shows period of illumination. Note no off-response by *L*-unit, but small spikes from other units are seen, together with small off-wave of illumination potential. Graph shows discharge rate plotted against time.

Firm evidence for the existence of synapses on the pathway from the ommatidia to the single neurones studied was afforded in experiments in which small amounts of nicotine (0·1% nicotine base in mammalian Ringer's solution) were injected into the optic lobe. This resulted in complete abolition of responses of the single units to all forms of stimulation, although some spontaneous activity in other parts of the optic lobe still persisted for a time.

Anatomical location of the responding neurones

In Fig. 1 are shown the numbers of responses observed at each of a series of depths alongside a scale diagram of the optic lobe. The responses

fall into two groups, one centred round 1·1 mm and a second centred round 1·5 mm from the surface. The peak of the more superficial group corresponds to the second synaptic region, and that of the deeper group to the third synaptic region. In view of the large size (10–15 μ) of the neurone somata in relation to the very thin non-myelinated fibres in the optic lobe, and the dimensions of the recording electrode (10 μ tip), it seemed highly probable that the recorded responses originated from the somata rather

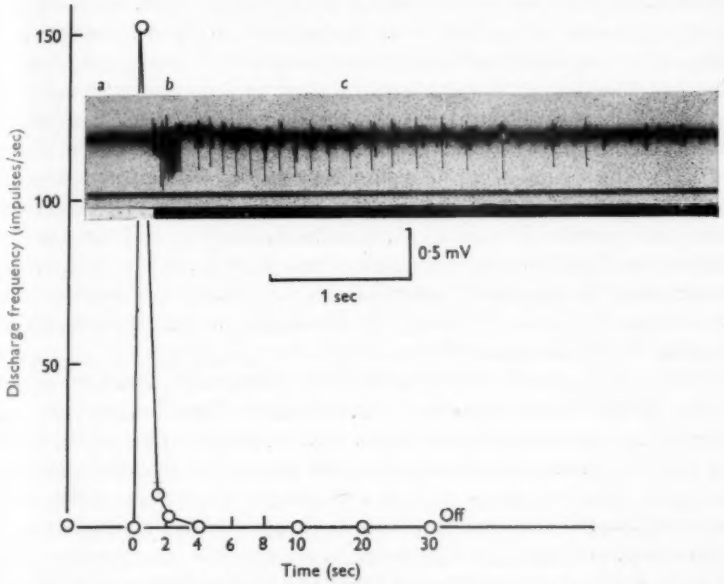

Fig. 6. Oscillogram shows behaviour of an on-unit. Note *a*, no 'dark' discharge; *b*, on-response; *c*, brief train of spikes. Inversion of spikes is of instrumental origin. Graph shows discharge rate plotted against time.

than the fibres. It will be seen from Fig. 1 that most of the neurone somata are situated laterally, between the synaptic regions, although a few are found scattered across the width of the lobe at the same levels. These neurones do not form a morphologically homogeneous population: some (Fig. 1, *a*) send a short process to terminate in an arborization in the second synaptic region; others (Fig. 1, 2–3) send out a longer process, which after forming an arborization in the second region terminates in a further arborization in the third region. We are not at present able to correlate these anatomical differences with specific types of single-unit behaviour. Situated between the second and third regions are other groups of somata. Some (Fig. 1, *t.f.*) send out two processes, one forming an

arborization in the second region and the other passing as a fibre in a tract which proceeds directly into the optic stalk. Others (Fig. 1, *b*, *c*) send arborizing processes into the second or third regions.

In order to have further confirmation of the location of the single units the optic lobe was exposed and the electrode brought up to the anterior or posterior lateral surface. Identification of the zone of neurones between the first and second synaptic regions was made easier by the fact that under strong illumination the outline of the second region could be seen as a large pale mass within the lobe. It was found in these experiments that large spike responses could only be obtained from the zones of neurone somata, although extended observations were not usually possible since preparations with exposed optic lobes survived only for a short time. Insertions of the electrode in the anterior and posterior lateral surfaces, medial to the second synaptic region, revealed large spike responses in the zones of neurones *t.f.* and *b*, *c* (Fig. 1). Single spikes up to 1 mV in amplitude were recorded from zone *t.f.*, and the discharge rate of these units was altered by illumination. They could be detected readily even with the electrode on the surface of the optic lobe. On the other hand, the activity of neurones *b*, *c* was unaffected by variations in illumination, and they appeared to discharge spontaneously.

Thus it is suggested that the single-unit responses described originate at two distinct levels; from the neurone somata lying between and to the side of the first and second synaptic regions, and from the neurone group *t.f.* (Fig. 1) situated anteriorly between the second and third regions of synapses. The situations of these two groups of active neurones help to clarify an earlier observation (Burtt & Catton, 1956) where in some cases there were two distinct levels for maximum response to movement stimuli, superficial and deep to the second synaptic region. This was in all probability a less sharply defined observation of the two sets of responses. Those cases where only a single maximum was found (the majority) could be explained as being due to a line of penetration which failed to encounter the group *t.f.*, readily understandable in view of the smallness and lateral disposition of this group.

DISCUSSION

No published work on single-unit spike responses from the insect eye, or from any arthropod eye except that of *Limulus*, has been found in the literature. There are extensive data on single-unit responses from various vertebrate eyes, but since these differ so completely in structure from the insect compound eye it seems profitless to undertake a full discussion of results on the vertebrate, although some comparisons will be made.

The compound eye of *Limulus* differs from that of arthropods generally in that the photoreceptor layer (retinula cells of the individual ommatidia) is widely separated from the associated synaptic regions, which in this genus are situated in the brain. In insects and most other arthropods the synaptic layers lie in the compact optic lobe, which lies in close apposition to the base of the compound eye. As a consequence, the post-retinal fibres, which connect the photoreceptor layer to the first region of synapses in the optic lobe, are very short; in *Limulus* they are very long and together form the 'optic nerve'. In insects the fibres which pass in the optic stalk from the optic lobe to the brain are of at least the third order, and carry impulses which are the outcome of complex nervous activity in the synaptic regions. They may therefore be presumed to have lost some of the simplicity of relation to visual stimulation which may be expected of neurones more closely associated with the retinula cells.

Single-fibre records obtained from the optic nerve of *Limulus* (Hartline & Graham, 1932) were for long believed to represent activity in first-order fibres, originating directly from the retinula cells of individual ommatidia. More recent evidence (Waterman & Wiersma, 1954) suggests that at least one of the fibres from each ommatidium is in fact second-order, there being a synapse in the ommatidium itself, formed by a specially large retinula cell termed the 'eccentric cell'. The fibres from the eccentric cells are thicker than those from the normal retinula cells and the evidence suggests that the single-unit spikes recorded in *Limulus* optic nerve are derived solely from these large eccentric-cell fibres. The single-unit studies on *Limulus* eye led to the conclusion that they showed a uniform behaviour, characterized by an on-response, a discharge rate increasing with increase of light intensity and the absence of an off-response (Hartline & Graham, 1932). Their properties therefore closely resemble those of the *L*-units in the locust optic lobe described in the present work. Units giving a dark discharge and an off-response are found only in the centrally-placed optic ganglion situated in the brain of *Limulus* (Wilska & Hartline, 1941).

In making comparisons with the vertebrate eye it is reasonable to make the assumption that the synaptic layers of the insect optic lobe find their functional correlates in the zones of synapses in the retina. Until recently single-unit activity in the vertebrate eye has been recorded only from the ganglion-cell layer, e.g. Barlow (1953) or from single optic-nerve fibres (Hartline, 1938; Thomson, 1953), which would be expected to display similar properties. The records obtained in these ways have established the presence of three types of response; those giving an on-burst with a sustained discharge during illumination; those giving on- and off-bursts only; and those giving an off-burst followed by a brief dark discharge. Hartline (1938), recording from frog optic nerve fibres, found 30% on-units, 50%

on–off units and 30% off-units. Barlow (1953), recording from ganglion cells of the frog's retina, found 6% on-units, 50% on–off units and 44% off-units. Compared with the properties of single units in the frog's eye, the locust *L*-unit and on-unit appear to correspond to the frog on-unit, and the locust *D*-unit to the frog on–off unit, with the reservation that the latter has not been described as giving a discharge in darkness. The relative proportions in locust eye are 22% on-type units (*L*-units plus pure on-units) and 78% on–off (*D*-units), pure off-units not having been observed in the locust compound eye. It is interesting to note that the simple eyes (ocelli) of the locust give rise to pure off-responses in the ventral nerve cord (Burtt & Catton, 1954); one might speculate that these organs in some way supplement the functions of the compound eyes in providing an off-signalling system.

More recently, with the application of the glass pipette micro-electrode to studies of retinal function, spike responses have been observed at various depths in the retina. Thus Brindley (1956) recorded spikes from both the outer ends of the rods and cones and from the inner nuclear layer of the frog's retina. Brown & Wiesel (1959) recorded single-unit spikes from the inner nuclear layer of the cat's retina, which gave a low-level dark discharge inhibited by light, thus resembling the locust *D*-unit, although on- and off-bursts were absent. Byzov (1959) recorded single-unit spikes, showing on-, on–off- and off-bursts, over a range of depths from 60 to 140 μ in the frog's retina. He concluded that they originated from the cells of Dogiel, which occupy a position in the visual pathway corresponding to that of the ganglion cells, but are more deep-seated anatomically. He found some neurones which gave a dark discharge inhibited by light.

Granit (1955) regards the off-discharge in vertebrate eyes as related to an inhibitory process in the retina, the units discharging at 'light off' being those inhibited by light. We should expect then that an off-response would be a feature only of those units, such as the locust *D*-unit, which are inhibited by light. Correspondingly those units whose activity was increased by light would give an on-discharge and little or no off-discharge (the locust *L*-unit). On the other hand, an earlier conception of the complete specificity of visual unit responses, such that given fibres could be classified as on- or off-responders under all conditions, must be modified in view of more recent findings in vertebrate eyes. Thus in the cat's retina there is a great variability in the ratios of the on and off peak discharges of given units, according to the light intensity and state of adaptation (Granit, 1948; Donner & Willmer, 1950). We have not been able to demonstrate such lability of behaviour in locust eye visual units.

SUMMARY

1. Responses from single neurones in the optic lobe of the locust have been recorded by the use of fine-tipped glass-shielded metal micro-electrodes.

2. The responding units were of three kinds; those (termed *D*-units) giving brief bursts of spikes at 'light on' and 'off', showing a dark discharge inhibited by light, and giving responses to movement stimuli; those (termed *L*-units) giving a brief burst of spikes only at 'light on', being silent in darkness and showing a discharge rate which increased with increasing illumination, and giving no movement responses; units which gave a pure on-response. Of 31 units studied, 24 were *D*-units, 5 were *L*-units and 2 were on-units.

3. The active units were identified as neurone somata associated with the second and third synaptic regions of the optic lobe. Their activity was abolished by nicotine.

4. The visual-field response area of the single units was much greater than that associated with a single ommatidium, and covered almost the whole visual field.

5. Parallels in behaviour between these units and single-unit behaviour in other eyes are discussed.

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Note added in proof. While this paper was in the press the authors' attention was drawn to a letter by Kuwabara & Noka (1959) on single-unit recordings from retinula cells in the eye of the insect *Lucilia*. These responses are of great interest but, being of the nature of relativity slow illumination potentials, are not directly comparable with the optic lobe spike responses described in the present paper.

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ACTIVE MUSCLE VASODILATATION PRODUCED BY STIMULATION OF THE BRAIN STEM: ITS SIGNIFICANCE IN THE DEFENCE REACTION

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The existence of a sympathetic vasodilator nerve supply to the skeletal muscles of the cat and dog has been known for some time (Bülbring & Burn, 1935; Folkow & Uvnäs, 1950). More recently it was discovered that these vasodilator nerve fibres can be activated by electrical stimulation in the hypothalamus, in regions of the mid-brain tegmentum and medulla, and, in the dog, by stimulation of a small region of the motor cortex (Eliasson, Folkow, Lindgren & Uvnäs, 1951; Eliasson, Lindgren & Uvnäs, 1952; Lindgren & Uvnäs, 1953; Lindgren, 1955). These findings led Lindgren, Rosén, Strandberg & Uvnäs (1956) to postulate the existence of a vasodilator pathway, originating in the anterior sigmoid gyrus and passing to the spinal cord via the hypothalamus, mid-brain tegmentum and medulla. They suggested that this pathway was involved in the cardiovascular adjustments of muscular exercise.

Previously, Eliasson *et al.* (1951) had noted that the muscle vasodilatation produced by hypothalamic stimulation was accompanied by vasoconstriction in the skin and intestine, and by other signs of sympathetic activity. In view of the known role of the hypothalamus in the expression of 'fear' and 'rage', they suggested that the vasodilator fibres were normally activated as part of the patterned response characteristic of these emergency reactions. According to the view of Eliasson, Lindgren & Uvnäs (1954), the hypothalamic areas were to be regarded as relay stations on a vasodilator pathway which could be activated not only in cases of emergency, but indeed whenever a sudden increase in muscle blood flow would be required for muscular effort.

In the conscious cat, systematic stimulation of points in the hypothalamus enabled Hess to define a region from which co-ordinated behaviour characteristic of the emergency reactions could be evoked (Hess & Brügger, 1943). This behaviour pattern, which commenced with alerting and culminated in flight or attack, was termed the defence reaction. In

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the present experiments we have located those parts of the hypothalamus and mid-brain from which the muscle vasodilatation is evoked and have shown that they are in fact the regions responsible for the whole integrated defence reaction in the conscious cat. Thus, strictly speaking, these are not vasodilator areas, but co-ordinating centres which produce muscle vasodilatation as a part of the defence reaction. In addition, we have located a separate vasodilator pathway which is activated by this extensive defence centre and which runs in close relation to the cerebral peduncles.

Woodworth & Sherrington (1904) showed that stimulation of peripheral nerves in the high-decerebrate cat elicited a response comprising many features of the defence reaction, which they termed the pseudaffective reflex. Cannon & Britton (1925) showed that all the autonomic features of the defence reaction which were known at the time could be produced reflexly in such preparations. In the present experiments, the active muscle vasodilatation has been found to be a prominent feature of these reflex responses, the reflex centre presumably being those regions of the hypothalamus and mid-brain from which the defence reactions are obtained.

METHODS

All experiments were performed on cats. The acute experiments were carried out under chloralose anaesthesia, a dose of 60–80 mg/kg being injected intravenously after anaesthesia had been induced with ethyl chloride and ether. To prepare a hind limb for registration of changes of muscle blood flow, a longitudinal incision was made in the skin along the inner side of the leg from the inguinal region to the ankle, the skin was separated from the underlying tissues, the circulation through the foot was occluded by a stout ligature round the limb just above the ankle joint and the skin was then sewn back over the limb. The femoral venous outflow was passed via polythene cannulae through a photo-electric drop-recorder and returned to the femoral vein more centrally. Changes of skin flow in the forepaw were registered similarly by recording the outflow from the cephalic vein. To record intestinal flow the circulation through several arcades supplying a section of the small intestine was isolated and the outflow from the appropriate branch of the superior mesenteric vein was passed through the drop-chamber and back into a branch of the portal vein. Heparin (1000 u./kg) was injected 1 hr after all dissections had been completed, and before the veins were cannulated for recording venous outflow.

Blood pressure was recorded by a mercury manometer connected to a carotid artery. Respiratory movements were registered by a piston recorder connected to corrugated rubber tubing held round the animal's chest.

Deep structures of the brain stem were stimulated by the use of stereotactically placed monopolar steel micro-electrodes. They were prepared from steel sewing needles by electrolytic erosion (Bishop & Collin, 1951) to a tip size of 5–20 μ, and were varnished except for the tip, either with a single coat of Dulux varnish, or with four coats of 'Araldite' 985E. The indifferent electrode was a similar steel needle pushed under the skin of the neck. A square-wave stimulator was used, the output being isolated from earth by means of an RF unit.

Electrolytic lesions were produced with similar electrodes, the insulation having been scraped off the tip to leave the last 0·5 mm bare. In some experiments, these electrodes were inserted in pairs, 2 mm apart, at the same antero-posterior level, and lesions were made by

passing current from one electrode to the other. In some, a single electrode was used, current being passed between this electrode and an indifferent electrode under the skin.

In a number of experiments electrodes were implanted into the brain stem in sterile operations under pentobarbitone anaesthesia. These electrodes were no. 6 or no. 4 intestinal needles made from austenitic steel and coated with 'Araldite' 985E. The needles used had a tip diameter of about 50 μ. The electrodes were implanted either singly or in assemblies. In such an assembly the needles, spaced at 2 mm intervals, were previously fixed in a small fluon (Polytetrafluoroethylene, Imperial Chemical Industries Ltd.) plate. The electrodes were implanted by means of the stereotactic apparatus. To aid fixation of the electrodes, 2 or 3 small (10 BA) stainless steel screws were screwed into the outer table of the skull, one being used for holding the indifferent electrode, a small stainless steel plate. Acrylic resin (Dental Fillings, Ltd.) was used to cover the electrodes and screws, and to cement them together. The leads were brought under the skin of the neck and out between the scapulae, to be fixed to a connector carried by the animal on a leather or polythene jacket.

Exact location of the needles was determined histologically at the end of each experiment. The brain was fixed by perfusion with alcoholic formol saline. Sections 25 μ thick were cut on the freezing microtome and stained with luxol fast blue and cresyl violet (Klüver & Barrera, 1953).

In some experiments muscle blood flow was registered in decerebrate cats. Under ether anaesthesia the calvarium was removed and the thalamus, basal ganglia and cerebral hemispheres were extirpated with a spatula. The anaesthetic was then discontinued. Two methods were used to record muscle blood flow. In one it was recorded directly by a cross-circulation technique. The circulation of one gastrocnemius muscle of the decerebrate cat was isolated and the muscle was supplied with blood from a heparinized donor cat. The venous outflow from the muscle was registered by the drop-recorder on its return to the donor. The spinal cord of the decerebrate cat was tied below the emergence of the L6 roots, so as to eliminate movements of the gastrocnemius while preserving its sympathetic innervation. The other method enabled the preparation to be more rapid and avoided the use of heparin. The changes of muscle blood flow were registered by a modification of the venous temperature method described by Abrahams (1959). In the original method a small chamber bearing a thermistor bead is inserted between the cut ends of the femoral vein. In the present modification cutting the vein was avoided by mounting the thermistor bead on the surface of a small strip of 'Araldite' which was held close to the external surface of the vein by an 'Araldite' cuff placed round the vessel. In this way adequate records were obtained of the changes in venous blood temperature which result from changes in muscle blood flow.

RESULTS

The finding of Eliasson *et al.* (1951) and Lindgren (1955), that active vasodilatation in skeletal muscle is elicited by electrical stimulation of regions of the hypothalamus and mid-brain, was readily confirmed. This vasodilatation usually appeared after about 5–10 sec of stimulation and reached a maximum within a few seconds. When stimulating with voltages just above the threshold (2–5 V under the present experimental conditions) the muscle blood flow often approached the value seen after maximal tetanic contraction. In some experiments, there was almost a tenfold increase in flow. As was shown by Lindgren (1955), square-wave stimulation at a frequency of 70–80 pulses/sec and with a pulse width of 2 msec is optimal for this response. With these parameters it was possible to

elicit reproducible responses from the same point in the hypothalamus at intervals of 3–4 min. We have elicited up to 13 successive responses without any obvious diminution.

At most points in the hypothalamus from which vasodilatation was elicited, the stimulation also produced moderate rises of arterial blood pressure, up to 40 mm Hg. On stimulation in some parts of the mid-brain these blood-pressure rises were much higher. It was clear, however, that these rises in blood pressure made little if any contribution to the increase in blood flow. Almost invariably the rise of blood pressure occurred before the onset of vasodilatation, and the pressure was back to normal when the vasodilatation was at its peak. Moreover, small doses of atropine (0·1 mg/kg) abolished or greatly reduced the vasodilator response (Eliasson *et al.* 1951), while the blood pressure rise then produced was if anything greater.

As was noted by Eliasson *et al.* (1951), the vasodilatation was usually accompanied by vasoconstriction in the skin and intestine, by tachycardia, pupillary dilatation and retraction of the nictitating membrane. In addition, we observed widening of the palpebral fissure, pilo-erection along the tail and mid line of the back, and changes in respiration. Respiratory movements were usually rapid, though much reduced in depth, the chest being held predominantly in the inspiratory position. The respiratory change made no significant contribution to the cardiovascular responses; for they persisted when the animals were artificially respired. With light anaesthesia stimulation often produced swishing movements of the tail, arching of the back and small movements of the jaws and limbs. In general, these associated effects were most prominent on stimulation of the brain-stem regions which produced the largest vasodilator responses.

Localization in the hypothalamus

The excitable area was sharply localized. As an example, the three panels in Fig. 1 show the effects of stimulating three points 1 mm apart, along a single electrode track in the tuberal region or the hypothalamus. The muscle vasodilatation obtained on stimulation at the middle point (marked 0) was much greater than that produced by stimulation 1 mm above or below it (marked X' and X). Figure 2 shows a hemisection of the brain from this experiment, in which there are four electrode tracks, the diagrammatic hemisection on the right illustrating the position of the electrode tip when the results shown in Fig. 1 were obtained. With the electrodes used stimulation probably only occurred close to the tip. From results such as these it was possible to construct maps of the restricted areas of the hypothalamus from which large vasodilator responses in skeletal muscle were obtained. The maps shown in Fig. 4 incorporate the results of twenty-two experiments in which the hypothalamus was

systematically explored, and of fifteen experiments in which electrodes were chronically implanted. At each level the results are included from planes 1 mm anterior and posterior to the level of section. These maps give a somewhat less precise localization than that actually obtained in individual experiments, because the borders of the area vary a little from

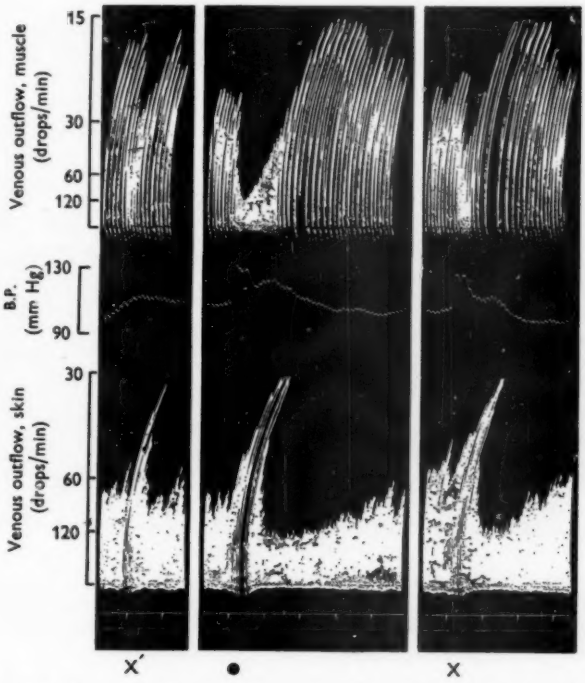

Fig. 1. Cat, chloralose. Records of venous outflow from skinned lower leg (top) and skin of the fore-paw (bottom), and of arterial blood pressure (B.P.). At X′, ● and X, stimulation at 3 successive points, 1 mm apart, in one electrode track in hypothalamus. Position of electrode tip at each point is shown in Fig. 2. Time marker, 30 sec.

one animal to another. The levels of the four illustrative sections through the hypothalamus are shown on the paramedian sagittal section in Fig. 3.

The hypothalamus anterior to the optic chiasma was explored in six experiments. Large vasodilator responses were obtained in three of them. The excitable region extended horizontally in the undifferentiated grey matter just above the base of the brain, from 1 to 3 mm lateral to the mid line, as illustrated in Fig. 4*A*. Smaller responses were obtained from

stimulation more dorsally, close to the anterior commissure. At the level of the optic chiasma the excitable area was sharply localized about 1–2 mm lateral to the third ventricle, and just dorsal to the chiasma itself (Fig. 4*B*). In the remaining three experiments this region was the most anterior from which large vasodilator effects could be obtained.

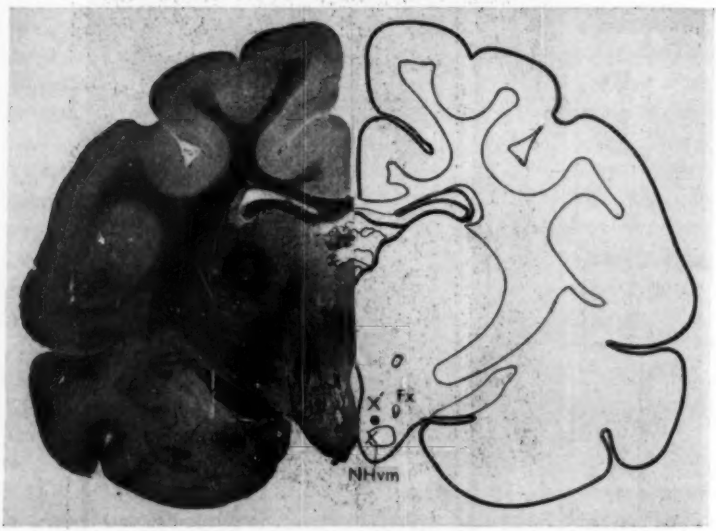

Fig. 2. Left: photomicrograph (coronal section) of cat's brain from same experiment as Fig. 1, showing four tracks left by stimulating electrode. Right: diagrammatic reproduction of section; X', ● and X indicate position of electrode tip when records of Fig. 1 obtained. Fx, fornix; NHvm, ventromedial nucleus.

On stimulation more posteriorly, at the tuberal level, the vasodilator responses were even larger. In all five of the animals in which this part of the hypothalamus was studied, the excitable area was in the perifornical region and extended laterally as a narrow band towards the zona incerta (Fig. 4*C*). In one animal the largest responses were obtained from a region dorsal to the fornix. Stimulation at the level of the mammillary bodies, carried out in five experiments, produced large responses from a strip dorsal and lateral to the mammillary bodies themselves. In four experiments, large responses were obtained from a region just dorsal to the cerebral peduncles (Fig. 4*D*).

Throughout the hypothalamus the most excitable region for these responses never appeared to be associated with any of the hypothalamic

nuclei. When stimulating in the tuberal region, for example, the vasodilator response was greatly reduced when the electrode tip passed into the ventromedial nucleus (as seen from Figs. 1 and 2). Likewise, stimulation in the mammillary bodies, supraoptic nucleus, paraventricular, suprachiasmatic and dorsomedial nuclei never produced a large vasodilatation. In different experiments the descending columns of the fornix were stimulated from the septal region to their termination in the tuberal region: the vasodilator effect was never pronounced.

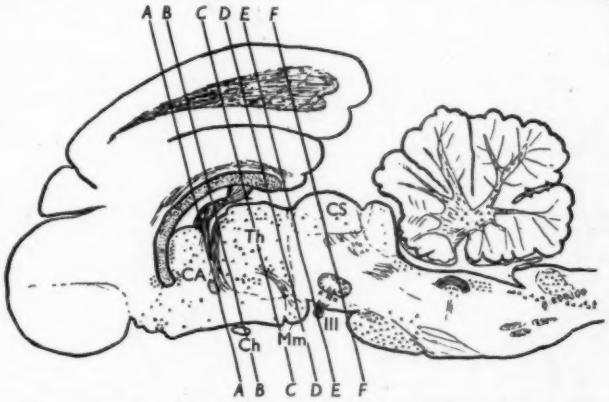

Fig. 3. Diagrammatic paramedian sagittal section of cat's brain indicating levels of coronal sections in Figs. 4 and 5. CA, anterior commissure; Ch, optic chiasma; CS, superior colliculus; Mm, mammillary body; Th, thalamus; III, oculomotor nerve. Section reproduced by permission of Editor of *Brain*.

Thus, the excitable zone in the hypothalamus consists of a narrow strip in the anatomically undifferentiated grey matter on both sides of the mid line, and extending through the whole length of the hypothalamus. In the tuberal region, where the largest responses are obtained, a lateral extension of the zone is apparent. A little more posteriorly, in the region of the mammillary bodies, this leads to a small, sharply localized area just dorsal to the cerebral peduncle, from which large responses are obtained.

Localization in the mesencephalon

An exploration was made posterior to the mammillary bodies, as far back as the intercollicular level, in 22 acute experiments and in 7 experiments with chronically implanted electrodes. As illustrated in the diagrammatic sections *E* and *F* of Fig. 5, the most readily excitable regions were in the tegmentum ventral to the superior colliculus (explored in 9 experiments), in the dorsolateral part of the central grey matter (explored in 10 experiments) and in a restricted area dorsal to each cerebral peduncle (explored

Fig. 4. Diagrammatic coronal sections showing regions in cat's hypothalamus from which active muscle vasodilatation is obtained on electrical stimulation. Hatching indicates regions from which vasodilatation regularly obtained. Dots indicate sites of large responses obtained in individual experiments. Sections *A*, *B*, *C* and *D* are at levels indicated in Fig. 3. Results are included from explorations in planes 1 mm anterior and posterior to each level of section. CA, anterior commissure; Ch, optic chiasma; Fx, fornix; Mm, mammillary body; Ped, cerebral peduncle; RPO, pre-optic area.

in 14 experiments). From these three regions, which appear to be well defined, large vasodilator responses were regularly obtained. Stimulation of the tegmentum between these regions sometimes also produced large vasodilator responses, but the excitable points were scattered and more difficult to find, and they varied from one animal to another. In Fig. 5*E* and *F* the dots indicate the points from which vasodilator responses were

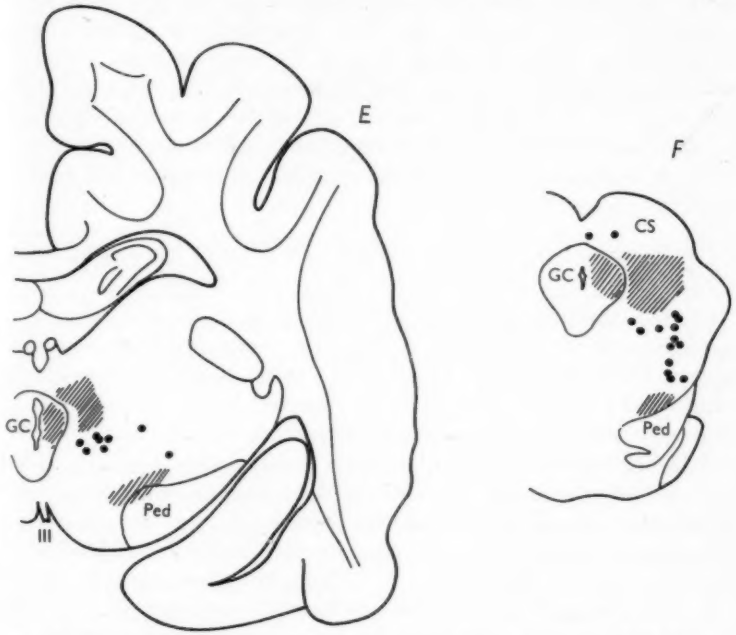

Fig. 5. Diagrammatic coronal sections showing regions in mesencephalon from which active muscle vasodilatation is obtained on electrical stimulation. Hatching indicates regions from which vasodilatation regularly obtained. Dots indicate sites of large responses obtained in individual experiments. Sections *E* and *F* are at levels indicated in Fig. 3. Results are included from explorations in planes 1 mm anterior and posterior to each level of section. CS, superior colliculus; GC, central grey matter; Ped, cerebral peduncle.

obtained in one or another experiment. These points may well be the sites of tracts which connect the central grey matter and tegmentum with the peduncular region.

At the level of the habenular nucleus, vasodilator responses could be elicited on stimulation of points in the pre-tectal region, presumably an anterior extension of the excitable region in the tegmentum ventral to the superior colliculi.

The separation of the three main regions is based not only on the ease with which the largest vasodilator responses were obtained, but also on differences of the associated autonomic effects. The effects produced from the central grey matter resembled those seen on hypothalamic stimulation: there was pronounced pilo-erection, pupillary dilatation, retraction of the nictitating membranes, and a moderate rise in arterial blood pressure and heart rate. The response to stimulation in the tegmental region was characterized by a particularly large rise of arterial blood pressure, often exceeding 200 mm Hg and associated with bradycardia. On stimulation of the peduncular region the responses differed from those of the other two regions, in that the associated effects were minimal: pilo-erection and retraction of the nictitating membranes were hardly ever seen and arterial blood pressure either fell or rose only very slightly.

Effect of mesencephalic lesions on active muscle vasodilatation produced by hypothalamic stimulation

The fact that stimulation of the peduncular region resulted in a large vasodilatation with few of the other accompanying autonomic effects suggested that this region, already seen at the level of the mammillary bodies, and extending through the mesencephalon in the same position relative to the cerebral peduncles, is the main efferent pathway to the medulla for the muscle vasodilatation. Direct evidence for this view was obtained by examining the effect of discrete mid-brain lesions on the vasodilator response to hypothalamic stimulation.

In these experiments a stimulating electrode was introduced into the excitable region at the tuberal level of the hypothalamus and fixed at a point from which large vasodilator responses were obtained. Two lesion electrodes were then introduced into the mesencephalon. As this pair of electrodes was racked down, electrolytic lesions were made at all points on each track where vasodilator responses could be obtained. The adequacy of each lesion was tested by passing the stimulating current again. If a vasodilator response was still obtained, the lesion was extended. Histological examination showed most of the lesions to be less than 1 mm in diameter.

After each lesion had been made the effectiveness of stimulating the hypothalamic point was tested. In only one experiment did the response to hypothalamic stimulation remain greatly reduced after a tegmental lesion had been made, and in this case the lesion electrode had produced a large haemorrhage occupying the whole of one colliculus and the underlying tegmentum. In 6 other experiments, even after multiple lesions in the superior colliculi, the tegmentum underlying them and the central

grey matter, the response to hypothalamic stimulation was much the same as it had been at the beginning of the experiment. There was often a transient reduction of the response, but this lasted for a few minutes only. Results from an experiment are shown in Fig. 6. The mid-brain section at the top shows some of the lesions made in four electrode tracks. Each track extended through the whole tegmentum, the lesions being made at all points where vasodilator responses were obtained. At the bottom are shown the vasodilator response to hypothalamic stimulation before (*a*) and 1 min after (*d*) a tegmental lesion had been made. The lesion was made at the upper end of the section of the track seen just to the right of the central grey matter. Stimulation at this point produced strong vasodilator and pressor responses before the lesion (*b*) and practically no response after it (*e*). Althogether 9 lesions were made during the course of this experiment and after all of them the hypothalamic response remained substantially the same. In striking contrast with the results of experiments such as this, a single small lesion restricted to the peduncular region from which vasodilator responses were obtained always abolished the hypothalamic response in 3 consecutive experiments. A typical result is shown in Fig. 7, in which the hypothalamic response was abolished by the lesion seen in the section.

Stimulation of the brain stem in the conscious cat

Defence reactions are obtained in conscious cats on stimulation of a well-defined region in the hypothalamus (Hess & Brügger, 1943). Similar reactions have been obtained from a localized region of the central grey matter (Hunsperger, 1956) and from points deep in the colliculi (Spiegel, Kletzkin & Szekely, 1954). From our experiments in anaesthetized cats the regions concerned appeared to be identical with those from which active muscle vasodilatation is most readily obtained. This identity was demonstrated in experiments in which electrodes were implanted in the hypothalamic and mid-brain areas which had been located on the basis of the vasodilator responses. Except in those animals with electrode implanted in the peduncular region, subsequent stimulation in the conscious cat gave a fairly constant pattern of behaviour. Threshold stimulation produced an alerting, or orientation, reaction. The resting animal would raise its head and look around, with moderate pupillary dilatation. As the voltage was increased, the animal would stand up with increasing pupillary dilatation and pilo-erection of the tail and back. It then began to hiss and snarl, with ears flattened and claws unsheathed. Finally, the cat would move rapidly around the observation chamber or would suddenly attack, particularly in response to a visual or auditory stimulus. The optimal stimulation parameters were the same as those for muscle vaso-

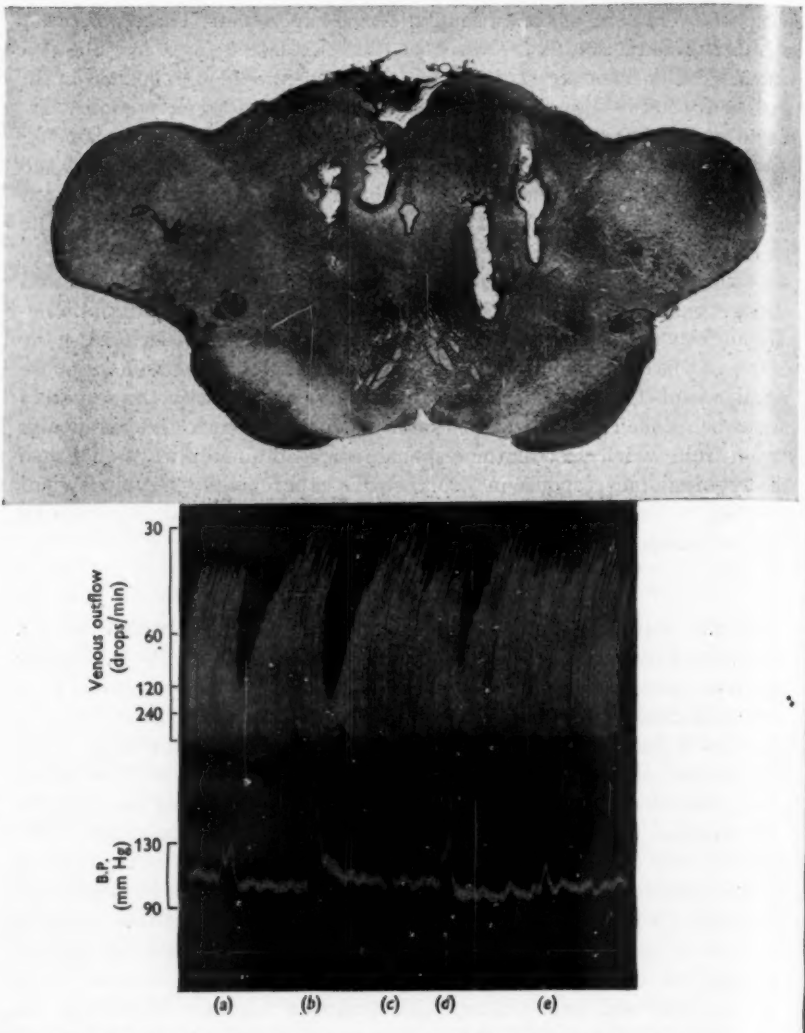

Fig. 6. Top: photomicrograph (coronal section) of cat's brain at level of superior colliculus, showing electrolytic lesions in four electrode tracks. Bottom: records of venous outflow from skinned hind limb and of arterial blood pressure. At (*a*) and (*d*), electrical stimulation of same hypothalamic point. At (*b*) and (*e*) stimulation at same point in mesencephalic tegmentum, at top of electrode track just on right of central grey matter. At (*c*) electrolytic lesion made at this mesencephalic point. Time marker, 30 sec.

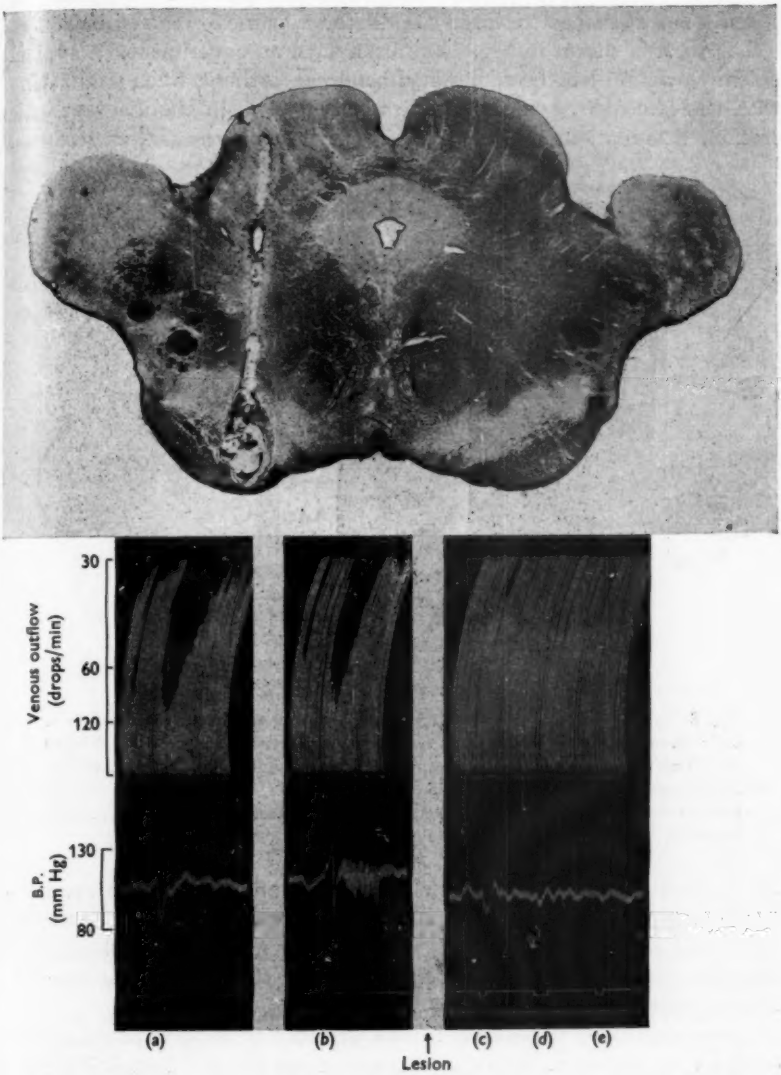

Fig. 7. Top: photomicrograph (coronal section) of cat's brain at level of superior colliculus, showing single electrolytic lesion in substantia nigra and cerebral peduncle. Bottom: records of venous outflow from skinned hind limb and of arterial blood pressure. At (*a*) and (*e*), stimulation of same point in peduncular region. At (*b*), (*c*) and (*d*), stimulation of hypothalamic point. Lesion made at peduncular point between (*b*) and (*c*). Time marker, 30 sec.

dilatation. When these animals were anaesthetized with chloralose and stimulation was repeated, large vasodilator responses were obtained.

The records shown in Fig. 8 are from such an experiment. A typical defence reaction had been obtained before anaesthesia when stimulating with the electrode in the pre-optic region, shown in the diagrammatic section. Stimulation under chloralose with the same parameters produced

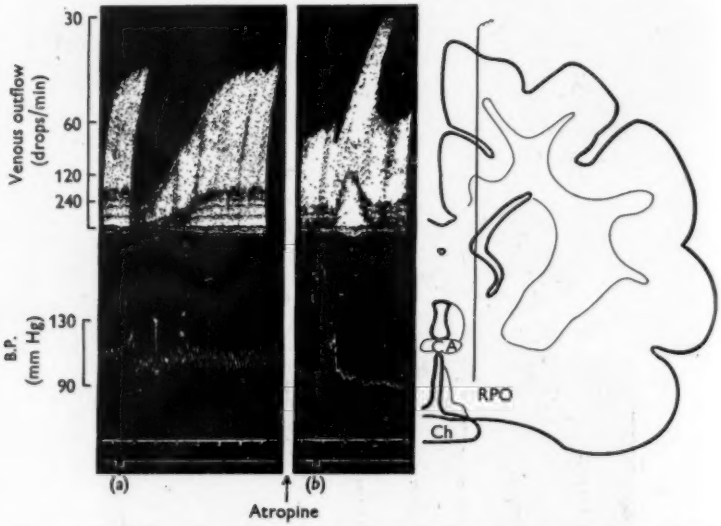

Fig. 8. Right: diagrammatic coronal section showing site in pre-optic region of cat's brain of implanted electrode. Left: records of venous outflow from skinned hind limb and of arterial blood pressure of same cat under chloralose anaesthesia. At signals, stimulation through implanted electrode before (*a*) and after (*b*) intravenous injection of atropine (0·5 mg/kg). CA, anterior commissure; Ch, optic chiasma; RPO, pre-optic area. Time marker, 30 sec.

the large muscle vasodilatation shown at (*a*) and, after an intravenous injection of atropine, the vasoconstriction shown at (*b*). With electrodes implanted in the hypothalamus, central grey matter and subcollicular tegmentum, the cardiovascular response and the behavioural reaction always went hand in hand.

From electrodes implanted in the peduncular region, however, the complex, co-ordinated, behavioural reaction was never obtained. The only responses seen in the conscious cat were either flattening of the homolateral ear, closure of the eye or rolling over on one side, whereas under anaesthesia the typical muscle vasodilatation was obtained.

It is concluded that the excitable regions on the hypothalamus and

central grey matter, and in the tegmentum ventral to the colliculi, function as integrative centres from which the component parts of the defence reaction, including muscle vasodilatation, are activated. These regions are shown as shaded areas in Fig. 9, which was constructed from our results. The areas in the hypothalamus and central grey matter are probably continuous. The posterior borders of the mid-brain areas have been left indefinite, since we have not explored further back in the brain stem, but from the reactions obtained by Keller (1932) and Bard & Macht (1958) with chronic decerebrate cats it appears that the areas might extend even into the pons. The vasodilator outflow from the integrative centres lies in the pathway just dorsal to the cerebral peduncles, indicated in Fig. 9 by the heavy interrupted line.

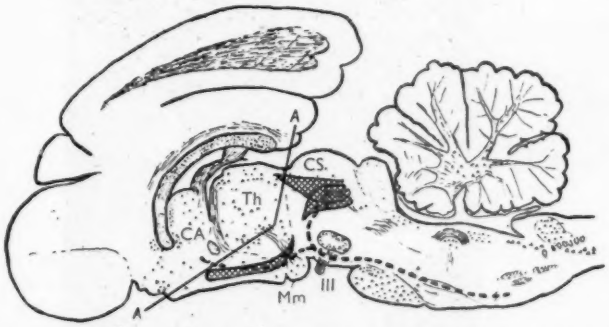

Fig. 9. Diagrammatic paramedian sagittal section of cat's brain. Cross-hatched areas represent regions in hypothalamus, central grey matter and mesencephalic tegmentum apparently functioning as reflex centres for the defence reaction. Heavy interrupted line represents vasodilator pathway connecting the centres with the periphery. *A–A* is the line of section used for decerebration. CA, anterior commissure; CS, superior colliculus; Mm, mammillary body; Th, thalamus; III, oculomotor nerve. Section reproduced by permission of Editor of *Brain*.

Reflex activation of the cholinergic vasodilator fibres to muscle in decerebrate preparations

In common with other reactions integrated at the diencephalic level, the defence reaction cannot be obtained as a reflex response in anaesthetized animals. Even under light chloralose anaesthesia, sciatic nerve stimulation will not produce any of the co-ordinated movements characteristic of the reaction. Of the autonomic responses, only the rise of arterial blood pressure and pupillary dilatation remain. We have never obtained an increase in muscle blood flow, and sometimes even saw a slight decrease.

The results in decerebrate preparations are different. Woodworth & Sherrington (1904) showed that sciatic nerve stimulation elicited a complex

reflex response in cats decerebrated at the pre-collicular level. This response, which they called the pseudaffective reflex, comprised pupillary dilatation, vocalization, snarling, movements of the limbs and a rise of blood pressure. As is seen from Fig. 9, decerebration at the pre-collicular level would leave intact the major part of the integrating region for the defence reaction.

We carried out experiments in which decerebration was started at the pre-collicular level and then extended rostrally to spare as much as possible of the hypothalamus, as indicated by the line on Fig. 9. In these preparations, electrical stimulation of the skin of one forelimb elicited, in addition to the effects described by Woodworth & Sherrington (1904),

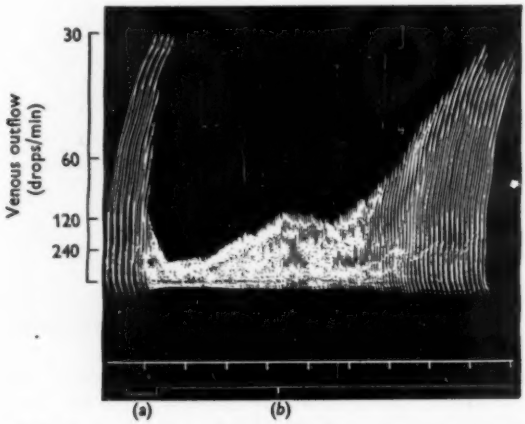

Fig. 10. High-decerebrate cat. Record of venous outflow from gastrocnemius muscle, perfused by donor cat. At (*a*) electrical stimulation of skin of forepaw. 'Spontaneous' reaction occurred at (*b*). Time marker, 30 sec.

arching of the back and pilo-erection. We did not always observe all parts of the reaction. This is in keeping with a similar finding of Cannon & Britton (1925) on partially decorticate cats. In 5 out of 9 such preparations skin stimulation also produced a large and prolonged increase in muscle blood flow. This is illustrated in Fig. 10, which is taken from an experiment in which the gastrocnemius muscle was perfused by a donor cat and its participation in the reflex movements was prevented by tying the spinal cord below L6. Pseudaffective reactions, including muscle vasodilatation, frequently occurred in the absence of a specific stimulus: an effect on muscle blood flow of such a spontaneous reaction is also illustrated in Fig. 10. In three of the cats a simplified preparation was used in which changes of muscle blood flow were registered indirectly, using an external

venous thermal recorder. In these preparations consistent pseudaffective responses were obtained for an hour or two and the muscle vasodilatation could then be shown to be abolished by small doses of atropine (Fig. 11).

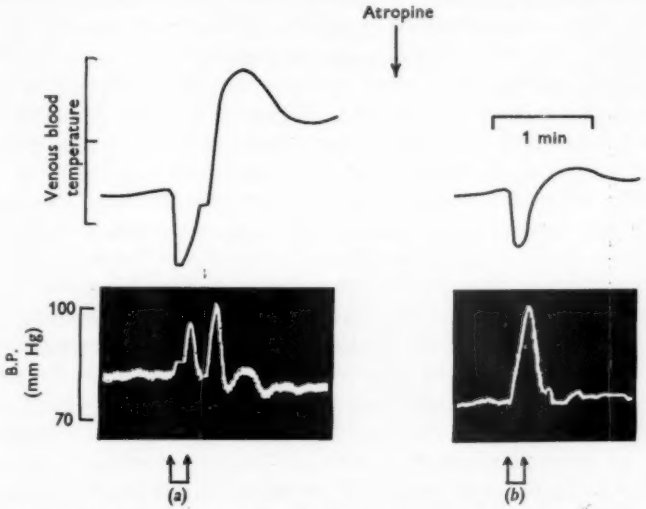

Fig. 11. High-decerebrate cat. Records of temperature of venous effluent from gastrocnemius muscle (upward deflexion signifies increase in venous outflow) and of arterial blood pressure. Each scale division on temperature record indicates 0·05° C. At signals, electrical stimulation of skin before (*a*) and after (*b*) intravenous injection of atropine (0·5 mg/kg).

DISCUSSION

Localization of the areas in the diencephalon and mesencephalon from which the cholinergic vasodilator fibres to skeletal muscle are activated has made it possible to draw several conclusions with regard to the organization of the integrated defence reaction in the brain stem. The hypothalamic area was seen to be strikingly similar to that defined by Hess, from which defence reactions are obtained in the conscious cat (Hess & Brügger, 1943). Indeed, stimulation by electrodes implanted in the 'vasodilator' area by means of the same parameters as those required for optimal vascular effects produced typical defence reactions ending in 'flight' or 'fight'. Even under an anaesthetic electrical stimulation produced all the features of the defence reaction which the preparation was capable of displaying. The pupillary dilatation, retraction of the nictitating membranes, pilo-erection and respiratory changes were almost always observed, and, if the anaesthesia was light, movements of the jaw, limbs

and tail were also seen. It thus appears that the muscle vasodilatation produced by stimulation of the hypothalamic area is really part of the whole defence reaction, which is integrated in this region of the brain stem.

Regions other than the hypothalamus were also found to possess this function. Active vasodilatation in muscle is produced by stimulating those parts of both the mid-brain tegmentum ventral to the colliculi and the central grey matter from which defence reactions are elicited in the conscious animal (Spiegel *et al.* 1954; Hunsperger, 1956). It appears from our results that these three brain-stem areas merge into one another, and we have not found any major functional distinction between them. Some small differences in one or another of the effects obtained from them on electrical stimulation were observed, the most consistent being the much larger rises of blood pressure obtained from the mid-brain tegmentum than from the hypothalamus or central grey matter. These large pressor reactions preceded the changes in regional blood flow, and therefore appear to result from an increase in cardiac output. Such an increased cardiac output, together with the vasodilatation in skeletal muscle and the vasoconstriction in the intestine, are well known components of the cardiovascular response observed during heavy muscular exercise, and can be understood as essential parts of the adjustments by which the circulatory system is prepared for the demands of sudden, severe muscular effort. Without this preparation, such exercise would rapidly entail a great increase in blood flow through all the muscles concerned, which would then have to be followed by widespread haemodynamic changes. It may therefore be of great advantage for the early period of such a response, if the vasodilatation, together with the circulatory adjustments necessary to maintain it, were to occur before the extensive muscular reactions have to take place. The pressor response obtained on stimulation of all these brain-stem areas (Kabat, Magoun & Ranson, 1935) would thus be seen, not as an event primarily concerned with regulation of arterial blood pressure, but as one feature of the defence reaction.

When Lindgren (1955) first showed that muscle vasodilatation could be elicited from points in the mid-brain tegmentum ventral to the colliculi, he concluded that he was stimulating the vasodilator outflow from the hypothalamus. This conclusion was based on an experiment in which the vasodilator response to hypothalamic stimulation was abolished by extirpating the inferior and superior colliculi on the same side. In another experiment undercutting the superior colliculus on the same side, and removing a large part of the subjacent tegmentum, reduced the vasodilator response to between a half and a third. In our experiments very small lesions in the mid-brain tegmentum often reduced the response to hypothalamic stimulation, when tested immediately after the lesion had been

made. However, a few minutes later the response was fully restored. This sensitivity to the acute effects of such lesions probably explains Lindgren's results (1955). By contrast, we found that small lesions restricted to the area above the cerebral peduncles from which vasodilatation could be elicited, regularly and irreversibly abolished the response to hypothalamic stimulation. We conclude that the true vasodilator pathway lies in this peduncular region. To support this conclusion were the additional findings that stimulation of the peduncular vasodilator region under an anaesthetic produced very little in the way of associated autonomic responses, sometimes only muscle vasodilatation with a small fall in blood pressure; and, even more striking, that in the conscious animal it never produced the co-ordinated defence reaction so characteristic of the other regions.

This peduncular vasodilator pathway, though most clearly seen as a separate entity at the mid-brain level, is also discernible in the posterior hypothalamus at the level of the mammillary bodies. As the excitable zones for the defence reaction in the hypothalamus, central grey matter and mid-brain tegmentum all connect with this peduncular pathway, there is every indication that this is the efferent pathway for the vasodilator component of the defence reaction. Thus our results support the hypothesis of a vasodilator pathway, although its course through the diencephalon and mesencephalon is not located as Lindgren *et al.* (1956) had thought. The more caudal extension of the pathway in the medulla was described by Lindgren & Uvnäs (1953) as a narrow longitudinal band lying 3–4 mm from the mid line and 1–2 mm above the ventral surface of the brain stem, and this is probably a direct continuation of the peduncular pathway in the mid-brain.

The pathway for the other cardiovascular effects and for the release of adrenaline and noradrenaline from the adrenals (Grant, Lindgren, Rosén & Uvnäs, 1958) which accompany the muscle vasodilatation, must be close to the vasodilator pathway; for all the cardiovascular effects of hypothalamic stimulation are abolished by the peduncular lesions. This result with such small lesions contrasts greatly with the finding of Magoun, Ranson & Hetherington (1938), who were unable to abolish the pressor responses to stimulation in the lateral hypothalamic area by extensive lesions in the mid-brain tegmentum. They concluded that the efferent pathway for these vasomotor responses is diffused through the whole mid-brain tegmentum. Our results with lesions restricted to the peduncular region show that specific and well-defined pathways do exist.

It is more than likely that the vasodilator points found in the anterior sigmoid gyrus and internal capsule (Eliasson *et al.* 1952) also connect directly with the peduncular pathway. This could provide the basis for activation of the same specific pattern of cardiovascular response in pre-

paration for any movement in which the sensori-motor cortex is involved, quite independently of the defence reaction. Indeed, Rushmer, Smith & Franklin (1959) have found that in conscious dogs electrical stimulation through an electrode implanted just dorsal to the peduncle, at the level of the mammillary bodies, produces a cardiovascular response similar to that of actual exercise without any external sign of distress.

It has been known since the classical descriptions of Goltz (1892) that the chronically decorticate animal readily displays defence reactions. In Goltz's dogs even quite trivial stimuli regularly and repeatedly produced a stereotyped pattern of aggressive behaviour, which was never modified as long as the animal lived. In other words, these behavioural reactions appeared as complex, co-ordinated reflexes. What Goltz (1892) could show in dogs, Bard & Rioch (1937) repeated in decorticate cats. 'Fear' and 'rage' reactions were regularly obtained in four such preparations, including one in which the thalamus and striatum had been removed in addition to the neocortex. Many years earlier, however, the pseudaffective reflex had been described by Woodworth & Sherrington (1904). This reflex response, which shows many features of the defence reaction, was obtained on electrical stimulation of the sciatic nerve in cats acutely decerebrated at the pre-collicular level. The stereotyped nature of this response to nociceptive stimulation enabled Woodworth & Sherrington (1904) to use it to trace the spinal pathways for pain. Their level of decerebration will have spared the mesencephalic section of the integrative region for the defence reaction and perhaps the posterior part of the diencephalic section. In our experiments, in the attempt to spare as much as possible of the whole brain-stem region, decerebration, though started at the same pre-collicular level, was extended further rostrally as the line of section approached the base of the brain. Sciatic nerve stimulation in such preparations produced more features of the defence reaction than were seen by Woodworth & Sherrington (1904). Moreover, we obtained evidence of active muscle vasodilatation which could be abolished by atropine. This is a direct demonstration that the muscle vasodilatation is an integral part of the whole co-ordinated defence reaction.

All these results lead us to conclude that the regions in the diencephalon and mesencephalon in which the response is integrated may be regarded, together, as the reflex centre for the reaction. The centre for the defence reflex would thus extend further caudally than was indicated by Bard's (1928) original decerebration experiments, which seemed to place it in the hypothalamus alone. It probably extends even into the pons; for Keller (1932) obtained typical 'rage' reactions in response to mild stimuli, such as pulling the skin of the back, in cats chronically decerebrated just

rostral to the beginning of the pons; and Bard & Macht (1958) obtained many features of the defence reaction in response to electrical stimulation of the tail, even when decerebration was carried out so far caudally as to remove the rostral part of the pons.

It is known that all the sensory systems have extensive connexions with the mesencephalic region in which we find part of the defence centre to be situated (Dell, 1952; French, Verzeano & Magoun, 1953). Evidence is accumulating that the undifferentiated grey matter of the hypothalamus should be regarded as a forward extension of this region. Of particular relevance is the recent demonstration by Nauta & Kuypers (1958) that fibres originating in the central grey matter and adjacent mid-brain tegmentum, which correspond to the more caudal part of the defence centre, project to the paramedian region of the hypothalamus and pre-optic area, which correspond to the more rostral part. It is therefore not surprising that electrophysiological techniques have shown sensory projections ascending not only to the sub-thalamic region of the diencephalon (Starzl, Taylor & Magoun, 1951; French, von Amerongen & Magoun, 1952), but also the hypothalamus itself (Ingvar & Hunter, 1955; Feldman, Van der Heide & Porter, 1959). Abrahams, Hilton & Malcolm (1959) have found that evoked potentials can be recorded in the cat in all parts of the defence centre in response to cutaneous stimulation. Responses to light flashes are almost as extensively found, and responses to clicks a little less so. In nature the full defence reaction is readily produced by nociceptive stimuli; but a sudden, strong stimulus of any modality will first evoke the alerting, or orientation reaction, which may develop into the full defence reaction if stimulation is sufficiently intense, and this is just the sequence of events seen on direct electrical stimulation of the defence centre. Hence the anatomical and electro-physiological evidence is in complete accord with the concepts put forward of a high-level centre for a complex behavioural reflex, elicited most readily by noxious stimuli but capable of reflex initiation by stimuli of all modalities. It is striking that the defence centre occupies such a large part of what has become known as the reticular activating system; for this emphasizes once again that, far from being merely a diffuse, non-specific system, a large part of this region of the mesencephalon and diencephalon has specific functions in the normal, unanaesthetized animal.

SUMMARY

1. In anaesthetized cats regions have been mapped in the hypothalamus, mesencephalic tegmentum and central grey matter from which active, atropine-sensitive muscle vasodilatation is elicited on electrical stimulation.

2. These three brain-stem regions are not merely 'vasodilator' areas, for the muscle vasodilatation is accompanied by other autonomic effects including a rise in arterial blood pressure, vasoconstriction in skin and intestine, pupil dilatation, retraction of the nictitating membranes and pilo-erection, by respiratory changes and movements of the jaw, limbs and tail.

3. Stimulation of all three brain-stem regions in the conscious cat through implanted electrodes produces co-ordinated defence reactions. It is concluded that the muscle vasodilatation is one component of the integrated response.

4. An additional, restricted region has been located just dorsal to the cerebral peduncles, stimulation of which produces active muscle vasodilatation in anaesthetized cats, and, in conscious cats, single or unorganized movements.

5. Lesions in the region dorsal to the cerebral peduncles abolish the vasodilator response to hypothalamic stimulation. It is concluded that the pathway for muscle vasodilatation runs in this peduncular region.

6. In the high-decerebrate cat, cutaneous stimulation reflexly produces many of the features of the defence reaction, including active muscle vasodilatation. It is concluded that the regions of the hypothalamus, mesencephalic tegmentum and central grey matter function as reflex centres for the co-ordinated autonomic and behavioural responses that comprise the defence reaction.

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SYNAPTIC POTENTIALS, AFTER-POTENTIALS AND SLOW RHYTHMS OF LATERAL GENICULATE NEURONES

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A detailed study has recently been made (Bishop & Evans, 1956; Bishop & Davis, 1960) of the recovery cycle of the sensory synapses in the lateral geniculate nucleus extending from absolute refractoriness till 5 sec after the discharge of the geniculate neurones. Following their discharge by an orthodromic volley, these neurones respond normally as soon as conduction becomes possible in the presynaptic fibres and, for a brief period while the presynaptic fibres are still recovering from refractoriness, they pass through a phase of relatively supernormal responsiveness. At shock intervals between about 3 and 6·7 msec the geniculate neurones pass through a phase of true supernormality, to be followed in turn by a deep and prolonged subnormality from which recovery is not complete until 2 sec have elapsed. In view of the well-known relationship which exists between the recovery cycle of peripheral axons and their after-potentials (Erlanger & Gasser, 1937) it was natural that we should attempt to record the after-potentials of the geniculate neurones and to examine the extent to which the recovery cycle of the geniculate synapses can be correlated with them. The morphological arrangement of the dendrites, cell bodies and axons of the principal cells in the lateral geniculate nucleus provides a favourable situation both for recording these after-potentials and for assigning electrical activity to histological structure. Vastola's earlier investigations (1957, 1959) of the after-potentials of geniculate neurones were largely concerned with antidromic activation. The present report is principally concerned with the after-potentials generated by orthodromic volleys, although antidromic activation has also been employed.

The ability to block transmission at junctional regions has for long been a powerful technique for studying synaptic mechanisms. In relation to the synapses in the lateral geniculate nucleus two new blocking techniques have recently become available, namely the use of the drug D-lysergic acid diethylamide (LSD) (Evarts, Landau, Freygang & Marshall, 1955; Bishop, Field, Hennessy & Smith, 1958; Bishop, Burke & Hayhow, 1959*b*)

and the phenomenon of post-tetanic delayed depression (Hughes, Evarts & Marshall, 1956; Evarts & Hughes, 1957*a*, *b*; Bishop, Burke & Hayhow, 1959*a*). When the synapses are blocked by these means the post-synaptic potentials produced by orthodromic volleys are strikingly similar to the after-potentials generated by the same neurones when normal synaptic transmission occurs and post-synaptic spikes are discharged. A detailed study was therefore made of the geniculate synaptic potentials produced under conditions of synaptic block, and this is now reported. The relatively small changes that take place in the synaptic potentials when normal synaptic transmission again becomes possible leads to the idea that the after-potentials as recorded have in them a very important component which is derived from the continued presence of the synaptic depolarization on parts of the neurone (i.e. dendrites) either subjected to prolonged action by the transmitter substance or possibly not normally invaded by the spike process. The available data relating to the synaptic potentials and after-potentials of spinal motoneurones will be reviewed from this point of view.

In this paper we also examined the relationship between the synaptic potentials and after-potentials of geniculate neurones and the spontaneous rhythmic potentials that may be recorded in the nucleus. Our results are interpreted as indicating that the spontaneous waves represent rhythmic fluctuations or the membrane potential of the dendrites and possibly the cell bodies of geniculate neurones. These apparently arise as a result of mechanisms intrinsic to the dendrites themselves although they can also be triggered off or modified both by orthodromic and antidromic volleys.

METHODS

Adult cats were anaesthetized with intraperitoneal allobarbitone solution (100 mg/ml., 0·5 ml./kg; Dial, Ciba) supplemented if necessary (usually at the onset) by the intravenous administration of small amounts (to a total of 0·3 ml., unless otherwise stated) of sodium pentobarbitone solution (64 mg/ml.; Sagatal, May and Baker). In most of the experiments the animals were also paralysed with gallamine triethiodide (Flaxedil, May and Baker, 8 mg intravenously at half-hourly intervals), in order to eliminate potentials of muscular origin, which can seriously distort the slower geniculate wave forms. Respiration was maintained by means of a pump. The body temperature was controlled with the aid of an electric heating blanket. The general methods used were the same as those described in previous papers from this laboratory (cf. particularly Bishop, Jeremy & Lance, 1953). After resection of the eyeball the optic nerve was suspended clear of orbital tissue. The optic nerve was stimulated electrically and the responses in and about the opposite lateral geniculate body were recorded by means of a stereotaxically directed steel micro-electrode introduced down through the intact cerebral cortex. All the potentials were recorded extracellularly between the micro-electrode and an 'indifferent' electrode inserted into the occipital muscles.

Steel micro-electrodes are subject to slow drifts in potential due to polarization effects, though these are minimized by the high-impedance input stage of our amplifiers. A control experiment, however, with the glass micro-pipette type of electrode, showed that the form

and time course of the slow geniculate wave forms were the same with either method. In the earlier experiments the uninsulated tips of the steel micro-electrodes were coated with platinum black, but this was discontinued when it was shown that the wave forms were not changed by its use. The deposition of platinum black is, however, a useful method for determining the extent of the uninsulated tip of the micro-electrode. The steel micro-electrodes were preferred because they are more convenient for stereotaxic use, they have a low resistance and the recording site can be easily labelled for subsequent histological identification by the electrolytic deposition of iron. Glass micropipette electrodes offered no advantage, because the geniculate responses were always recorded against a background of biologically generated 'spontaneous' slow waves of potential, which completely mask any polarization effects. These base-line fluctuations were offset by recording and measuring large numbers of traces. One stage of condenser-coupling was used in the amplifiers, but the time constant chosen was always shown to be adequate by recording the responses with a still longer time constant.

The geniculate neurones were activated antidromically by embedding a bank of 12 steel micro-electrodes in the gyrus lateralis of the cerebral cortex. These stimulating electrodes, similar to those used for recording, were arranged in two parallel rows of six each, with the rows 2 mm apart and the individual electrodes in a row also 2 mm apart. The rows were positioned along the gyrus with the centre of the bank at approximate Horsley–Clarke co-ordinates of 3·5 mm lateral and 7·5 mm anterior and the tips of the electrodes 3–4 mm below the surface of the cortex among the terminating optic radiation axons. The recording electrode was usually located in the anterior half of the lateral geniculate body and histological studies in this laboratory (W. R. Hayow & C. Webb, unpublished) have shown that the stimulating electrodes were placed in a part of the cortex to which this portion of the geniculate projects (cf. Doty, 1958). Any combination of six pairs of stimulating electrodes could be chosen by means of six switches and, by trial and error, the combination was found which gave a large response with the least distortion due to stimulus escape. The stimulus artifact was further reduced by means of a balancing circuit.

In the experiments with D-lysergic acid diethylamide (LSD-25, Sandoz) the drug was introduced in the carotid stream by retrograde injection into the lingual artery. For details of the method see Biahop, Field, Hennessy & Smith (1958).

RESULTS

The details of the notation used to describe the various geniculate potentials are given in earlier publications from this laboratory (cf. Bishop & Evans, 1956). The two optic tract spikes and their corresponding post-synaptic spikes are designated t_1 and t_2 and r_1 and r_2, respectively. The corresponding synaptic potentials are s_1 and s_2 respectively and further subscripts of r (r_3, r_4, etc.) are used to refer to the repetitive waves (Fig. 1, *a*) that frequently follow the r_1 spike (Bishop, Jeremy & McLeod, 1953). The recording sites were usually situated in the anterior portion of the lateral geniculate body and the crossed optic pathway was nearly always used.

In determining the duration of the various slow post-synaptic potentials it has already been pointed out (see Methods) that the major source of error is due to interference from the background of 'spontaneous' slow waves of electrical activity. This may be largely offset by measuring a sufficiently large number of records. There are, however, other difficulties,

both theoretical and practical. When the afferent volley is restricted to t_1 fibres, the commencement of post-synaptic activity is clearly defined and there is therefore no difficulty with respect to the time of onset of potentials which are subliminal for a propagated response (synaptic potential (s_1), Fig. 5, *a*). The time of onset of the after-potentials which follow the propagated spike is however rather indefinite. Furthermore, both the synaptic

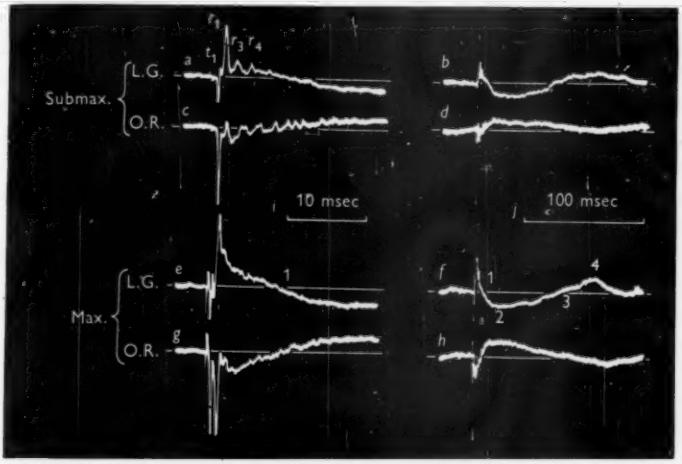


Fig. 1. Responses recorded in the lateral geniculate nucleus (L.G.) and in the optic radiation (O.R.) following single shocks applied to the contralateral optic nerve. t_1, presynaptic spike; r_1, r_2, r_4, post-synaptic spikes. Submaximal shock is 65% maximal for t_1. Maximal shock is maximal for whole optic nerve (t_1 and t_2). Numerals associated with traces *e* and *f* indicate how the time course of the after-potentials was measured. Time scale *i* refers to traces *a*, *c*, *e* and *g*, and time scale *j*, to traces *b*, *d*, *f* and *h*. Upward deflexions in this and subsequent records indicate negativity of micro-electrode.

potentials and the after-potentials associated with t_1 volleys are of such a low amplitude that their subsequent time course is also rather uncertain. Increasing the stimulus strength to include t_2 fibres increases the amplitude of the slow post-synaptic potentials, making them much easier to measure, but at the same time distorting their time course because of the later onset of the post-synaptic activity associated with the more slowly conducting group of afferent fibres. The times of onset of these later-arising potentials are also much more uncertain, not only because they are superimposed on potentials generated by the t_1 volley but also because the wave forms themselves may be difficult to interpret. This is particularly the case when the recording site is near the anterior pole of the lateral geniculate nucleus (Fig. 1, *e*). Although not many t_2 fibres project to the anterior

portion, those that do are among the fastest of the t_2 group, so that t_2 and r_1 spikes may be practically synchronous.

Because of these uncertainties measurements were usually made both with submaximal (t_1 only) and maximal optic nerve shocks. For practical purposes it was decided to make all measurements with respect to the stimulus artifact and subsequently to subtract the appropriate latency to arrive at the correct time course. It was decided also to regard the after-potentials as commencing at the same time as the synaptic potentials, i.e. before the spike potential in each case. With a t_1 volley the mean latency of the s_1 potential was 1·14 msec, but when the shock strength became maximal this latency shortened to 1·05 msec. With increasing shock strength the stimulating current spreads more proximally, so that the latency of the t_1 volley decreases from about 0·85 to 0·75 msec. The mean latency of the t_2 post-synaptic activity was 2·22 msec, corresponding to a t_2 volley latency of about 1·7 msec. In order to arrive at the correct time course for the synaptic potentials and after-potentials associated with a maximal optic nerve volley it was decided to subtract 1·6 msec from the measured values in each case, this being the mean of the latencies of the t_1 and t_2 post-synaptic potentials (i.e. 1·14 and 2·22 msec respectively).

In summary, therefore, all measurements were made with respect to the stimulus artifact and either 1·1 or 1·6 msec was subtracted, depending upon whether the stimulus was submaximal or maximal. This correction is, of course, only of significance in respect to the first 10–20 msec of post-synaptic activity. No systematic attempt was made to measure the amplitudes of the potentials.

After-potentials in lateral geniculate body

Systematic recordings were made in the nucleus at thirteen sites in eleven preparations. Responses to maximal optic nerve shocks were obtained in every case and, at seven of the recording sites, submaximal shocks were also used. Typical wave forms (labelled L.G.) are shown in Fig. 1; *a* and *b* are due to a submaximal shock and *e* and *f* to a maximal shock. The submaximal shock was, in this instance, about 65% maximal for t_1. The after-potentials are seen to consist of a sequence of oscillations of potential, initially negative. These oscillations may continue for half a second or more (Fig. 8) until they gradually become indistinguishable from the slow waves of the background activity. The times of the significant features in the course of the after-potentials (and also the synaptic potentials, see below) have been measured at the times indicated by the numerals in Fig. 1, namely: (1), at the end of the first negative potential; (2), at the maximum amplitude of the first positive wave; (3), at the end of the first positive wave and (4), at the peak of the late negative wave (see

Tables 1–3). It was usual to record the wave forms at the two time-base durations of approximately 30 and 170 msec respectively so that the first and later portions of the response could be measured accurately. Each response was recorded between 10 and 50 times, the stimuli being applied at 5 sec intervals.

The small repetitive waves (r_3, r_4, Fig. 1, *a*) superimposed upon the first negative after-potential are due to the approximately synchronous repetitive firing of a considerable number of geniculate neurones (Bishop *et al.* 1953). While repetitive firing is a constant feature of the geniculate response, particularly in relation to the large fibres at low levels of stimulation, it is not usually so prominent as that shown in Fig. 1. These repetitive waves are nearly always sharply limited to the duration of the first negative after-potential, and it is obviously difficult to decide how much of the latter is true after-potential process and how much of it is due to summed asynchronous repetitive firing. The repetitive firing may be obscured or possibly eliminated when large stimulating shocks are used (Fig. 1, *e*). The relationship of the repetitive firing to the negative after-potential is shown more clearly in Fig. 2. Trace *a*, which is due to a shock submaximal for t_1, has been obtained on one preparation, and *b* and *c* from another preparation. In the latter instance maximal stimulation was used, the traces being recorded successively at the same site at different time-base sweep speeds. The repetitive firing stops sbruptly in every case as the trace goes positive. The relationship of repetitive firing to the slow negative potentials that may be recorded from the region of cell bodies has been observed by several investigators (e.g. Baumgarten & Jung, 1952; Tasaki, Polley & Orrego, 1954; Li, Cullen & Jasper, 1956). Strong support for the view that the slow negative potentials in the lateral geniculate nucleus act as a generator potential for the repetitive firing has been provided by single-unit studies in this laboratory (P. O. Bishop, W. Burke & R. Davis, unpublished).

The negative after-potential associated with a t_1 volley is usually not very prominent and may be obscured at its onset by a transient reversal of current flow that follows the discharge of the r_1 spike as the dendritic-cell body membrane becomes a source of current for the spike in the axon. By the time that the negative after-potential can again be recorded it may then be of very low amplitude. It is to be expected that increasing the shock strength would increase the amplitude of the after-potentials without very significantly altering their time course. Inspection of Fig. 1 and Table 1 shows that this is the case. The first negative after-potential has a duration slightly over 7 msec. The subsequent positive after-potential has a sharp decline to a maximum positivity at about 22 msec, thereafter returning rather more slowly to the base line at about 80 msec. The positive

after-potential is devoid of any evidence of repetitive firing. The late negative wave is usually more symmetrical in form, having a peak soon after about 100 msec (see below). Associated with this peak there is frequently a repetitive discharge of geniculate neurones, seen as unit spikes in Fig. 8, *b*, *c*. While the late negative wave was present in every experiment it was not always prominent. The subsequent oscillations of potential are difficult to distinguish from background activity. It should be stressed that occasionally the positive after-potential failed to regain the base line and occasionally also the tracing, although it crossed the base line, had not achieved a late peak of negativity by the time it reached

TABLE 1. Duration of after-potentials (msec)

(*a*) Recording in lateral geniculate nucleus

Afferent volley	No. of recording sites	1*	2	3	4
t_1	7	7·0 (4·1–12·3)	25 (21–37)	70† (51–96)	95† (89–119)
t_1 and t_2	13	7·3 (4·2–9·8)	22 (10–30)	80† (49–116)	100† (75–160)

(*b*) Recording in optic radiation

Afferent volley	No. of recording sites	1*	2	3	4
t_1	2	7·1	24 (17–31)	80† (64–91)	95† (84–102)
t_1 and t_2	6	8·6 (7·1–10·9)	33 (18–41)	90† (67–114)	100† (88–116)

The values given are mean values, the range of variation being given in parentheses.

* These numbrals refer to the labelled wave forms in Fig. 1. † These figures are underestimates to the extent that certain records have been neglected (for explanation see text).

the edge of the cathode-ray tube screen. In arriving at the mean figures given above and in the preparation of the various tables these records were neglected. For this reason certain values in the tables have been labelled as approximate. The peak of the late negative wave therefore occurs somewhat later than the value of 100 msec given above.

There is probably no subliminal fringe associated with a maximal optic nerve volley, and the subliminal fringe associated with a submaximal t_1 volley is quite small (Bishop & Davis, 1960). The potentials described above can, therefore, be regarded as being generated in neurones all of which have been brought to discharge by the afferent volley.

After-potentials recorded in optic radiation

The cells in the lateral geniculate nucleus are arranged in sharply defined and fairly closely packed layers, and the axons issue from the dorsal surface, proceeding upwards to the cerebral cortex. At the upper surface of the nucleus, therefore, the axons above are fairly sharply

separated from the cellular elements of the nucleus below. The potential fields in the nucleus will be determined very largely by currents flowing into or out of dendrites and cell bodies, though the fields will be modified by the presence of the initial portions of the optic radiation axons among their cells of origin. Above the nucleus, on the other hand, the flows of current will be associated only with axons. A study of the distribution of the flows of current associated with such a favourable histological arrangement provides valuable clues to the nature of the after-potentials and the structures responsible for their production. Bishop (1953) and Bishop & McLeod (1954) have already made a study of the distribution of synaptic currents, particularly in relation to blocked geniculate synapses, during the first 5–10 msec after the arrival of the afferent volley. Vastola (1957, 1959) has also carried out similar investigations in relation to the slow after-potentials following both orthodromic and antidromic activation. An earlier report (Bishop, Burke, Davis & Hayhow, 1958) and the results reported here amplify and extend these investigations.

After-potentials were systematically recorded at six sites in five preparations about 1 mm above the dorsal surface of the nucleus, and with both submaximal and maximal optic nerve shocks. The records (labelled O.R.) shown at *c*, *d*, *g* and *h* in Fig. 1 are fairly typical, the submaximal shock being again 65% maximal for t_1. These records were actually obtained about 4 hr after traces *a*, *b*, *e* and *f* in Fig. 5, when the synapses had recovered from block caused by the drug LSD (for details see below). The presynaptic volley is now recorded as an entirely positive spike and the post-synaptic spike is here a low-amplitude initially-positive triphasic response. Except for the reversal in polarity, the after-potentials are very similar in form and time course to those recorded in the nucleus. They are, however, of lower amplitude and it was usually only possible to make satisfactory measurements when maximal optic nerve shocks were used. The times of occurrence of significant features of the wave forms, measured in the same way as in the case of the intra-nuclear responses, are given in Table 1 (*b*). The initial slow positive wave has a mean duration of 8·6 msec. The subsequent slow negative wave reaches a peak at about 33 msec and declines to the base line again at about 90 msec. The latter time and the late positive wave that followed were very variable and difficult to measure. Not infrequently the negative wave failed to regain the base line and the late positive wave was in fact only positive-going and not actually positive.

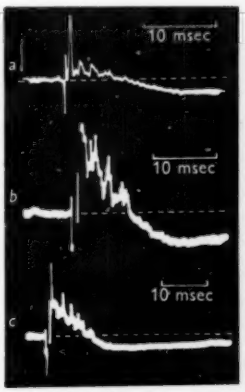
It has already been noted that, in the nucleus, repetitive spikes were superimposed on the initial negative after-potential and that unit spikes were also frequently recorded on the late negative wave. In the optic radiation these are again recorded but this time superimposed upon and

generally confined to the corresponding positive waves. The initial positive wave in response (*c*) of Fig. 1 is largely obscured by a train of repetitive spikes which is no longer evident when the optic nerve shock becomes maximal (Fig. 1, *g*). There can be little doubt that the repetitive waves in Fig. 1, *c* represent the nearly synchronous repetitive firing of single optic radiation axons, because we have recorded repetitive firing of this kind from single radiation axons with micropipette electrodes having tip diameters of 0·5 μ or less. The repetitive firing described above is very similar to the recurrent efferent discharge that may occur in a few motor axons following antidromic impulses in the same axons (Renshaw, 1941). This discharge is again limited to the slow negative phase of the antidromic potentials (Brooks, Downman & Eccles, 1950*a*).

As the micro-electrode is inserted down through the optic radiation into the lateral geniculate nucleus the after-potentials must at some stage reverse their polarity. A detailed study of this change in polarity was made in a number of preparations by recording the after-potentials at successive small intervals, both as the micro-electrode was inserted downwards into the nucleus and also on its withdrawal. The records shown in Fig. 3*A* were selected from such a series. At level (0) the trace is virtually isopotential throughout the time when after-potentials are recorded at sites above and below this level. In five recording series in four preparations this reversal level occurred at a mean depth of 12·0 mm from the surface of the cerebral cortex, but for descriptive purposes the reversal level will be regarded as zero. Thus the numerals in Fig. 3*A* indicate recording sites in millimetres above and below this level. The reversal point was necessarily somewhat arbitrary, since it was taken to be that level at which the oscillograph trace was most nearly isopotential taking into account interference from background activity. Approaching from above, the after-potentials appear between 2 and 3 mm above zero and increase in voltage until the slow negative wave reaches a maximum amplitude at a mean level of 0·9 mm above. Thereafter the potentials decline until zero level is reached. Just below this a low-amplitude slow positive wave appears which has an abrupt onset immediately after the end of the spike potential. On penetrating more deeply a small negative-going hump appears on the positive wave 6–8 msec after the start of post-synaptic activity; the hump grows in amplitude, helping to fill out and make negative the earliest portion of the positive wave at depths between 0·2 and 0·55 mm below zero. The after-potentials, negative and positive, thereafter grow in amplitude so that the positive wave reaches a maximum amplitude at a mean level of 1·2 mm below. On going deeper the amplitudes gradually diminish, again without change in form.

Although the pattern described above was usually observed, very

occasionally the small negative-going hump was observed at levels at and even above zero. In the centre trace of Fig. 4*A*, which was regarded as zero level, a very small negative hump can be observed immediately after



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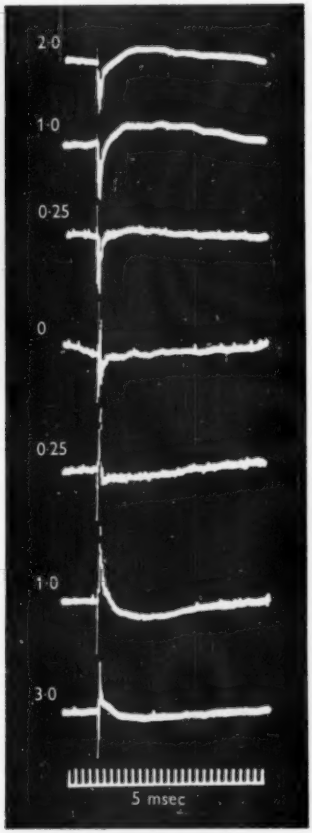


3A

3B

Fig. 2. Repetitive firing of geniculate neurones in relation to the negative after-potential. *a*, response following a single shock to the contralateral optic nerve which was submaximal for t_1; *b* and *c*, responses in another preparation following single shocks which were close to maximal for t_1 and t_2.

Fig. 3. *A*. Responses following single shocks applied to the contralateral optic nerve recorded, at distances indicated in mm, above and below the locus at which the trace labelled 0 was obtained. *B*. Outline drawing of a parasagittal section of the lateral geniculate nucleus showing recording site (●) used to obtain record labelled 0 in Fig. 3*A*; arrows indicate horizontal and vertical Horsley–Clarke planes.

the spike potentials. Rarely a similar but still negative-going hump has been recorded at levels above zero, where it appears on the initial positive wave. The significance of the negative-going hump and its relation to the negative after-potential is not clear. It is possibly due to a late post-synaptic propagated discharge from the lateral geniculate nucleus.

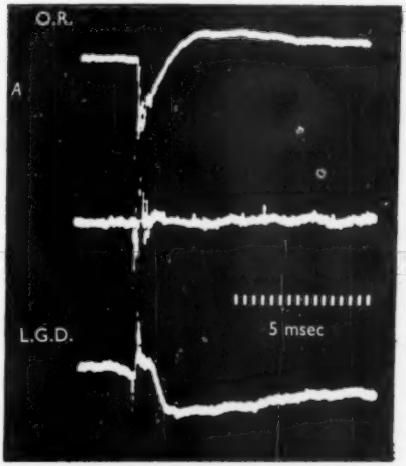
It was clearly important to determine the precise histological location of the tip of the micro-electrode at zero level and this was done by labelling the recording site with electrolytically deposited iron. Due allowance for slow-wave background activity is fairly readily made if the sequence of cathode-ray tube photographs is available for study. This was obviously not practicable and judgement concerning the location of the zero level had to be made by observing the cathode-ray tube screen at the time of the actual recording. Despite this uncertainty, the labelled sites were all very close to the upper surface of the lateral geniculate nucleus. The location of the zero level for the series in Fig. 3*A* is shown as a filled circle in the parasagittal outline drawing of the lateral geniculate body (Fig. 3*B*). Because of the larger amplitude of the after-potentials maximum stimulation was always used to locate the zero level. In one experiment a bank of three micro-electrodes was assembled with the shafts, 1 mm apart, in parallel array and the tips offset in depth, again 1 mm apart. The bank was gradually inserted vertically down through the cortex, with the plane of the electrodes parallel to the Horsley–Clarke anteroposterior plane, until the central electrode was judged to be at zero level. A series of recordings were then taken from the three electrodes (Fig. 4*A*) and then each site was labelled with iron. The labelled sites are shown in the micro-photograph (Fig. 4*B*) of a parasagittal section through the nucleus. The zero level is again at the upper surface of the cellular layers.

It is interesting to observe that at about the zero level the traces nearly always showed much greater evidence of unit activity and repetitive firing. Although the electrodes used were not suitable for unit recording, increased unit activity round the zero level is clearly evident in the traces in Fig. 3*A* and 4*A*, occurring along the whole length of the traces.

Synaptic potentials

Bishop & McLeod (1954) studied the synaptic potentials that may be recorded in and about the lateral geniculate body when synaptic transmission has been blocked. They showed that the synaptic potential produced by a t_1 volley is recorded as a negative wave in the nucleus, reversing to a positive wave when the recording site is in the optic radiation above the nucleus. Particular attention was given to the level of reversal of this potential which, in the case of a contralateral volley, was shown to be at, or very close to, the upper surface of the nucleus. At that time also it

was noted that the synaptic potential was followed by a prolonged after-positivity which was recorded as an after-negativity in the optic radiation. Further observations in this laboratory while studying the blocking action



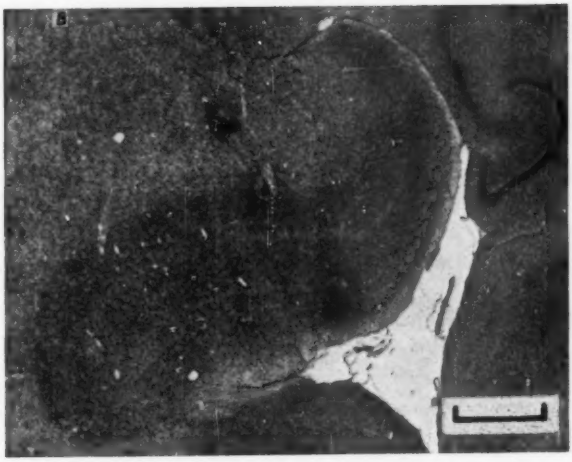


Fig. 4. *A*. Responses following single maximal shocks applied to the contralatera optic nerve and obtained by inserting three micro-electrodes simultaneously into the region of the lateral geniculate nucleus. O.R., optic radiation; L.G.D., lateral geniculate nucleus. *B*. Microphotograph of a parasagittal section through the lateral geniculate nucleus (Nissl stain). The recording sites used to obtain the records in Fig. 4*A* are indicated by the three 'blue' spots. Scale, 1 mm.

both of drugs and of prolonged repetitive stimulation confirmed these earlier findings, and the striking resemblance, both in respect to form and time course, between the synaptic potential and after-potentials as described above prompted a more detailed study of the synaptic potentials, particularly in respect to their later time course.

Bishop & McLeod (1954) used three different methods to obtain a synaptic block, namely asphyxia, deep pentobarbital anaesthesia and repetitive stimulation. The first two methods were clearly unsatisfactory. The last method, which entailed placing a test volley about 15 msec after a conditioning tetanus of 3–5 volleys at about 300/sec, is also unsatisfactory from the point of view of studying the form and time course of the synaptic potentials because they are superimposed upon and distorted by the after-potentials following the conditioning tetanus. Since then two further techniques have become available for producing a synaptic block in the lateral geniculate nucleus, namely the action of the drug D-lysergic acid diethylamide (LSD) (Evarts *et al.* 1955; Bishop, Burke & Davis, 1958; Bishop *et al.* 1959*b*) and the phenomenon of post-tetanic delayed depression (Hughes *et al.* 1956; Evarts & Hughes, 1957*a*, *b*; Bishop *et al.* 1959*a*). Both of these methods have the advantage that the post-synaptic neurone is probably not affected to any extent by the blocking action. LSD probably blocks synaptic transmission by competitive antagonism to the normal transmitter and the phenomenon of post-tetanic delayed depression is probably due to presynaptic changes. In studying the synaptic potentials produced by these means, two criteria were used to decide when synaptic transmission was blocked, namely, the absence of any spike-like discharges on the post-synaptic wave form and the absence of any cortical response. The cortical response was first sampled at a number of places and the site chosen for the monitor electrode was always the one which gave the maximal response. The cortical response was then used as a continuous monitor of the level of the geniculate block.

Although about 100 μg LSD injected into the carotid artery will normally block transmission through the lateral geniculate nucleus, recovery commences almost immediately. It was, in fact, difficult to maintain a complete block for the time necessary to take a sufficiently large number of records, and even with the much larger doses that were actually used (500 μg repeated at intervals of 10–20 min) the block was usually rather uneven. On the other hand, the phenomenon of post-tetanic delayed depression produces a very stable and prolonged block in transmission. This depression is produced by tetanic stimulation of the optic nerve for 15 sec using maximal stimuli at about 400/sec. Following the tetanus the geniculate response is depressed for a few seconds, may then be larger than

normal (1–2 min), and finally is markedly reduced, and a complete block may remain for a period lasting hours.

In a number of preparations the attempt was made to compare the after-potentials and the synaptic potentials by recording them both at the same locus, in the nucleus on the one hand and in the radiation on the other. As full recovery from a large dose of LSD may take several hours and the effects of tetanic stimulation are even more prolonged, it was not possible to take all the recordings at only two sites, the first in the nucleus and the second in the radiation. Figures 1 (*a–h*), 5 (*a–h*) and 6 (*g–j*), which were all taken from the one preparation, illustrate the procedure that was adopted. Records *a*, *b*, *e* and *f* in Fig. 1 were first obtained in the nucleus

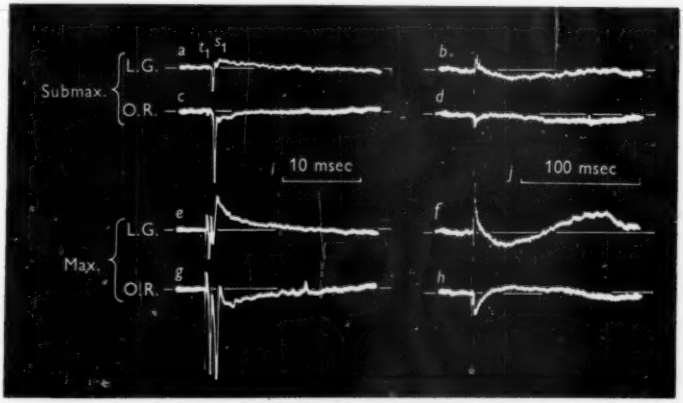


Fig. 5. Responses recorded in the lateral geniculate nucleus (L.G.) and in the optic radiation (O.R.) following single submaximal and maximal shocks applied to the contralateral optic nerve during LSD synaptic block. Same preparation and similar recording conditions as those used to obtain records in Fig. 1. t_1, presynaptic spike; s_1, synaptic potential. Records arranged as in Fig. 1. For details see text.

by using two strengths of stimulation which were thereafter kept constant throughout the experiment. The synapses were then completely blocked with repeated injections of LSD and the records *a*, *b*, *e* and *f* in Fig. 5 taken. About 4 hr later, when recovery from the drug was complete, the electrode was moved up into the optic radiation and records *c*, *d*, *g* and *h* in Fig. 1 were taken. Further amounts of LSD were then injected and records *c*, *d*, *g* and *h* in Fig. 5 were obtained during the height of synaptic block. Following partial recovery from the drug, a 15 sec tetanus was delivered to the optic nerve and, when complete synaptic block had been re-established, records were again taken at the radiation site (*i* and *j*, Fig. 6). The electrode was then returned to the nucleus as near as possible

to the site used to obtain the earlier geniculate responses and the records *g* and *h* of Fig. 6 were taken. Thus records *g* and *h* of Fig. 6 are not strictly comparable with the earlier geniculate records, though they are closely similar to them. It is interesting to note that after all these procedures in this experiment, a 10 min tetanus to the optic nerve, as is usual, restored the geniculate response to normal (cf. Evarts & Hughes, 1957*b*; Bishop *et al.* 1959*b*). The other traces in Fig. 6, taken from another preparation, also show the responses to maximal afferent volleys during post-tetanic delayed depression, in the nucleus (*a* and *b*) and in the radiation (*c* and *d*) respectively.

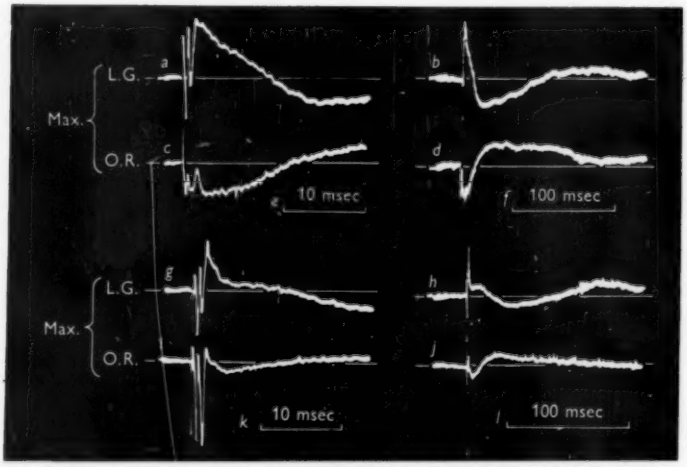


Fig. 6. Responses recorded in the lateral geniculate nucleus (L.G.) and in the optic radiation (O.R.) following single maximal shocks applied to the contralateral optic nerve during synaptic block obtained by post-tetanic delayed depression. Records *g*, *h*, *i* and *j* were obtained from the same preparation and under similar recording conditions as those of Fig. 1. Records *a*, *b*, *c* and *d* were obtained from another preparation. Each time scale refers to the two traces immediately above. For details see text.

The synaptic potentials found in both kinds of block were measured in the same way as the after-potentials and the results were pooled to produce Table 2. It can be seen that there is again relatively little difference between the responses due to volleys submaximal for t_1, and maximal nerve volleys. The initial negative wave has a duration of about 12 msec, the succeeding positivity reaches its maximum at about 25 msec, the base line is recrossed soon after 60 msec and the late negative wave has a peak at about 100 msec. Insufficient recordings have been made in the optic radiation for a satisfactory comparison between the time course of

the synaptic potentials in that site and in the nucleus. Except for the reversal in polarity the potentials are however very similar. The after-potentials and synaptic potentials recorded at the same locus are compared in Table 3. The initial negativity of the synaptic potential in both forms of block lasted consistently longer than the corresponding negativity of

TABLE 2. Duration of synaptic potentials (msec)

(*a*) Recording in lateral geniculate nucleus

Afferent volley	No. of recording sites	1*	2	3	4
t_1	5	11·8 (9·8–16·9)	33 (24–41)	70† (51–85)	100† (97–107)
t_1 and t_2	5	11·9 (8·9–16·1)	25 (18–33)	61 (40–92)	103 (96–111)

(*b*) Recording in optic radiation

Afferent volley	No. of recording sites	1*	2	3	4
t_1	2	8·6 (6·9–11·1)	37 (27–50)	68† (64–71)	95† (82–105)
t_1 and t_2	2	11·9 (11·0–12·9)	33 (27–40)	86	118 (112–128)

The values given are mean values, the range of variation being given in brackets.

* These numerals refer to the labelled wave forms in Fig. 1. † These figures are underestimates to the extent that certain records have been neglected (for explanation see text).

Note. At two sites in the nucleus and at one site in the radiation synaptic potentials were recorded both as a result of LSD and post-tetanic delayed depression.

TABLE 3. Duration of after-potentials and synaptic potentials (msec)

		1	2	3	4
A	After-potentials	7·7	22	68	101
	LSD synaptic potentials	13·1	23	47	98
B	After-potentials	8·5	23	74	103
	PTDD synaptic potentials	10·3	28	84	114

Mean values at times indicated by numerals (1–4) in Fig. 1.

the after-potential. There is, however, probably no significant difference between the responses as far as the time course of the remaining features of the waves is concerned. Earlier histological studies (Bishop & McLeod, 1954) established that the site of the reversal of polarity of the synaptic potentials was at the upper surface of the nucleus and this has been confirmed by further work in this laboratory using maximal optic nerve volleys (P. O. Bishop & R. Davis, unpublished observations).

Antidromically evoked after-potentials

The form of the antidromically evoked after-potentials is seen in Fig. 7. In our few experiments with antidromic stimulation measurements of the duration of the negative after-potential were uncertain because of distortion by stimulus escape and the difficulty in deciding when this escape

had been adequately controlled by the balancing circuit in the stimulus leads. In the case of the experiment illustrated in Fig. 7, with the stimulus artifact judged to be adequately controlled, the onset of the initial negative spike had a mean latency of 0·7 msec and the mean duration of the after-negativity was 4·9 msec (range 4·3–6·3) measured from the onset of the negative spike. Thereafter the trough of the after-positivity was reached at 16 msec, the base line was recrossed at 110 msec and the late negative wave had a peak at 150 msec. The after-positivity and later time course

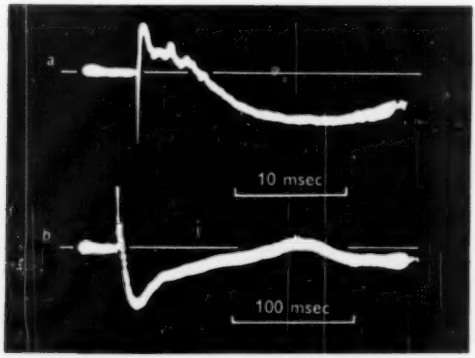


Fig. 7. Responses recorded in the lateral geniculate nucleus following antidromic volleys discharged down the optic radiation. The same response has been recorded successively with fast and slow time bases respectively.

of the orthodromic response were closely similar (Table 4*A*). The repetitive firing of geniculate neurones which occurs in response to antidromic volleys (Fig. 7, *a*) is again limited to the duration of the negative after-potential. This discharge almost certainly involves the repetitive firing of the same neurones, because the latter has actually been observed to occur when recording from single antidromically activated geniculate neurones (P. O. Bishop, W. Burke & R. Davis, unpublished). A similar antidromic repetitive discharge occurs in the ventral roots of the spinal cord (Renshaw, 1941; Brooks *et al.* 1950*a*; Granit, Pascoe & Steg, 1957). Our findings (Fig. 8) confirm Vastola's observation that the after-positivity in the antidromic responses corresponds to that seen in the orthodromic responses recorded at the same points. Records *a–g* of Fig. 8 were obtained from the same experiment as those of Fig. 7. Preliminary observations indicate that the antidromic after-potentials are unchanged by the drug LSD or during the period of post-tetanic delayed depression of the orthodromic response.

Slow geniculate rhythms

In seven preparations a study was made of the spontaneous slow oscillations of potential that may be recorded in the lateral geniculate nucleus. Except on one occasion both optic nerves were prepared for

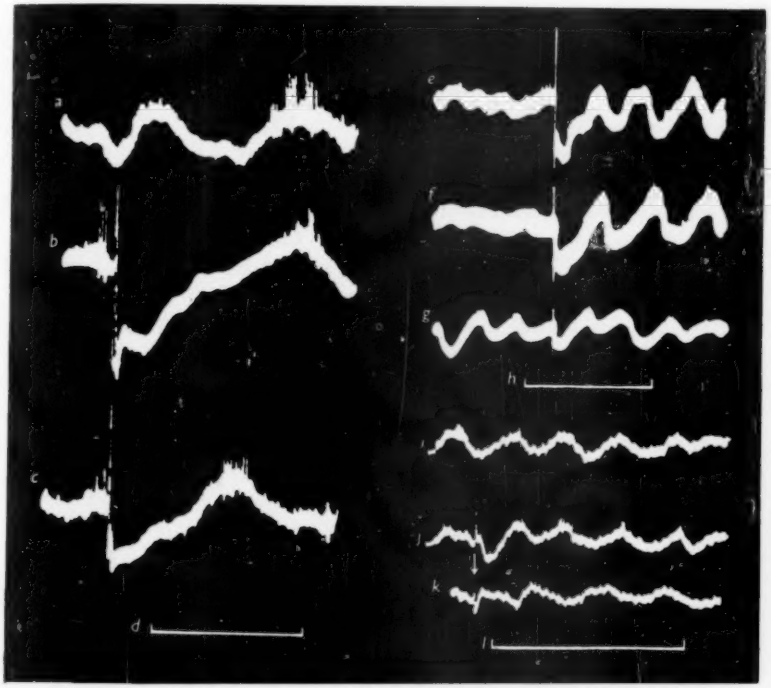


Fig. 8. Spontaneous and evoked slow rhythms. Traces *a–g* were obtained at the one site in the lateral geniculate nucleus. *a*, spontaneous rhythms; *b* and *e*, responses to maximal stimulation of the contralateral optic nerve; *c*, *f* and *g*, responses to antidromic volleys discharged down the optic radiation. *i*, *j* and *k* were obtained from another preparation during PTDD synaptic block, *i* and *j* being taken at a common site in the nucleus, and *k* just above the nucleus in the optic radiation. *i*, spontaneous rhythms; *j* and *k* responses to maximal stimulation of the contralateral optic nerve. Arrow indicates stimulus artifact in trace *k*. Each time scale refers to the three traces above it; *d*, 200 msec; *h*, 0·5 sec and *l*, 0·5 sec.

stimulation. Since this involves the removal of most of the retina and probably the inactivation of the small remnant, the spontaneous potentials do not depend upon retinal mechanisms. Recordings were taken about six or more hours after the induction of the anaesthesia (see Methods). The slow waves under these conditions are fairly irregular, being broken up

by fast activity, but there is a predominant rhythm between 6 and 15 c/s (mean 9 c/s; Fig. 8, *a*). Bursts of unit-spike activity also occur particularly in relation to the negative-going peaks of the waves. It is possible that the rhythmical bursts of repetitive firing that may follow single orthodromic volleys (Bishop, Burke, Davis & Hayhow, 1958) are also related to these spontaneous geniculate rhythms.

In four preparations the effect of a sudden increase in the depth of anaesthesia was studied. Within 1–2 min after the intravenous administration of 0·3–0·5 ml. sodium pentobarbitone solution the fast activity was markedly reduced and the predominant rhythm slowed to 3–9 c/s (mean 5 c/s). The waves generally increased in amplitude and the bursts of unit-spike activity were reduced (1 experiment) or eliminated (3 experiments).

The relationship of the spontaneous waves to those produced as a result of orthodromic and antidromic volleys has also been studied (Fig. 8). If either orthodromic or antidromic volleys are fired into the nucleus at a time when the spontaneous waves are minimal or absent, long-continuing oscillations of potential are produced which are indistinguishable from those arising spontaneously (Fig. 8, *e*, *f*). In these circumstances it is hard to avoid the conclusion that the mechanism responsible for the oscillations was activated by the arriving volleys. It is also obviously difficult to determine when triggered activity ceases and 'spontaneous' activity takes over. If we measure the first cycle of triggered activity from the stimulus artifact to the peak of the late negative wave (labelled 4 in Fig. 1) it has a mean duration of slightly over 100 msec (Table 1), corresponding to a frequency of about 9 c/s, the mean frequency of the spontaneous oscillations. When spontaneous oscillations of potential are occurring in the nucleus the arriving volley appears largely to halt this rhythm and to trigger it anew in phase with the stimulus (Fig. 8, *g*).

In one experiment a detailed comparison was made between the spontaneous rhythms and those produced by contralateral, ipsilateral and antidromic volleys, the latter three being again measured from stimulus artifact to the first late negative wave. The results (mean values) are shown in Table 4*A*. In the same experiment the later waves in the antidromic response were compared with the spontaneous rhythms recorded immediately beforehand, with an oscillograph time base permitting 3 or 4 later waves to be measured. The antidromically evoked waves had a mean frequency of 6·1 c/s, while that of the spontaneous waves was 6·2 c/s.

Spontaneous slow oscillations of potential also occur in the lateral geniculate nucleus, both when the synapses are blocked by LSD and during post-tetanic delayed depression (PTDD). In one experiment (Table 4*B*) the mean frequency of the spontaneous rhythms was 8·7 c/s, and some hours later repeated injections of relatively large doses of LSD were given

but the spontaneous rhythms still continued though at a slightly reduced frequency (6·9 c/s) despite the synaptic block. In the same experiment the duration of the first cycle of the response to orthodromic volleys remained virtually unchanged during subsequent LSD block and during PTDD.

A detailed comparison was made in one experiment during PTDD between the spontaneously occurring slow waves, as recorded in the nucleus (Fig. 8, *i*), and the slow waves following orthodromic volleys recorded both in the nucleus (Fig. 8, *j*) and in the optic radiation (Fig. 8, *k*). The time base used allowed 4 or 5 waves to be measured in each sweep.

TABLE 4. Relationship between spontaneous and evoked potentials in two preparations (*A* and *B*)

		Mean interval (msec)	Frequency (c/s)
A	Spontaneous rhythms*	145	6·9
	After-potentials† following a		
	Contralateral volley	155	6·5
	Ipsilateral volley	140	7·1
	Antidromic volley	160	6·3
B	Spontaneous rhythms*		
	Before LSD	115	8·7
	After LSD	145	6·9
	Responses† to orthodromic stimulation		
	Normal after-potential	125	8·0
	LSD synaptic potential	115	8·7
	PTDD synaptic potential	115	8·7

* Measured between peaks of rhythmic oscillations.
† Measured from stimulus artifact to peak of the first late negative wave.

The mean frequency of the spontaneous rhythms was 6·3 c/s, while the mean frequency of the evoked slow waves was 7·1 c/s, both in the nucleus and in the optic radiation. It can be seen from records *i* and *j* of Fig. 8 that the nuclear and optic radiation waves remain approximately 180° out of phase throughout almost the whole duration of the sweep. It is probable therefore that the optic radiation slow waves arise as a result of electrotonic propagation of oscillations of membrane potential from the cell body and dendrites of the geniculate neurones.

DISCUSSION

General considerations

In order that the electrical records obtained from cell populations may be correlated with histological structure a detailed knowledge is required of the geometrical arrangement of the functionally distinct portions of the neurone both within the individual neurones themselves and as between one neurone and another. The relevant information that is

available in relation to the lateral geniculate nucleus has already been discussed in earlier analyses of this kind (G. H. Bishop & O'Leary, 1942; Bishop, 1953; Bishop & McLeod, 1954; Vastola, 1957). To assist the present discussion it is desirable to emphasize some of the structural features of the principal cells, i.e. those giving rise to axons that proceed upwards in the optic radiation. The dendritic shafts arborize extensively in all directions away from the cell body (Taboada, 1927). This predominantly radial arrangement of the dendrites with respect to the individual neurone is however obscured in the population by the extensive intertwining of the dendrites from adjacent neurones. The general orientation of the dendrites is therefore essentially random. As far as the axons are concerned, from whatever part of the cell they arise they quickly achieve a roughly parallel orientation as they pass towards the upper surface of the nucleus and beyond into the optic radiation.

Considering the events in synaptic transmission it may be supposed that the impulses in the presynaptic endings lead to local depolarizations of the post-synaptic membrane. The presence of local sources and sinks of current on dendrites which are randomly arranged with respect to one another would not produce a potential field effective for recording. A post-synaptic potential would not therefore be recorded until the depolarization of the dendritic tree and cell body had become sufficiently widespread for significant currents to be drawn from the portions of the radiation axons towards the upper surface of the nucleus and beyond into the optic radiation. In this region the separation of axons on the one hand from cell bodies and dendrites on the other leads to a corresponding separation of sources and sinks of current and hence of positive and negative fields. Even considering a single cell the radial arrangement of the dendrites means that the initial portion of the axon will be electrically hidden, so that net outward flow of membrane current will be found only as the axon approaches and leaves the limits of the field of distribution of the cell's dendrites.

Several important consequences follow from the above considerations. It will not be easy to distinguish between dendritic and cell-body potentials, since both are recorded only by reason of electrotonic current spread up the radiation axons and the recording of dendritic potentials must necessarily involve the cell body. Furthermore, although there is reason to believe from a study of single-unit records that the initial segment of the axon has properties which distinguish it from other parts of the neurone (Freygang, 1958; P. O. Bishop, W. Burke and R. Davis, unpublished; cf. Eccles, 1957), it is likely that under the present recording conditions its contribution will be swamped by the potentials of dendritic and cell-body origin. Neither can the records described above provide any direct evidence for

conduction along dendrites, although it has been suggested on other evidence (Bishop, 1953; Bishop & McLeod, 1954; Freygang, 1958) that dendritic conduction probably does not occur in the lateral geniculate nucleus. Since the volume conductor records of dendritic and cell-body potentials are obtained because of the difference in potential which exists between the surface of the dendritic-cell body membrane and that of the axon, any potential changes that occur in the axon will have a distorting effect on the former potentials. These effects will be minimal when synaptic transmission is blocked but the presence of a spike and after-potentials in the axons will lead to important modifications of the dendritic and cell-body potentials.

The above considerations do not, of course, necessarily apply to extracellular recordings made with micro-electrodes having tip diameters of $0{\cdot}5\,\mu$ or less, since with their aid it is possible to record local intensities of membrane current associated with particular parts of individual cells to the exclusion of the general field potentials. For this reason extracellular records obtained with such micro-electrodes differ markedly from those obtained with the micro-electrodes used in the present study (Freygang, 1958; Bishop, Burke & Davis, 1958).

Synaptic potentials

It will be convenient to discuss the synaptic potentials first, since changes in the membrane potential of the post-synaptic axons are not directly involved in this case. Analysis of the spatial distribution of the sources and sinks of current indicates that the axonal changes occur only as a result of electrotonic spread of current from the cell body and dendrites. The currents arise as a result of oscillation in the membrane potential of the cell body and dendrites. As was pointed out above, our records do not allow separate cell-body and dendritic components to be distinguished, although it is highly probable that both structures are actively involved (cf. Fatt, 1957*a*, *b*; Curtis & Eccles, 1959; Rall, 1959). An initial depolarization (negative) phase, lasting about 12 msec, is followed by a hyperpolarization (positive) phase which has a trough at about 25 msec after the onset of post-synaptic activity. The base line is crossed again at about 60 msec, the late negative wave (peak at about 100 msec) leading on to further oscillations of potential which are difficult to distinguish from background slow-wave activity. It should be emphasized again that the mean values for the duration of the positive and the late negative waves given above are underestimates (p. 520).

Bishop (1953) and Bishop & McLeod (1954) noted that the initial post-synaptic negativity is followed by a long-lasting positivity, but the phenomenon was not further investigated at that time. The synaptic

potentials studied by Bishop & McLeod (1954) had a much faster time course than those examined here, presumably because they were produced by a test shock timed to coincide with the trough of the positive after-potential that follows a brief high-frequency train of conditioning shocks. Under these circumstances the synaptic potentials are distorted by the currents associated with the after-potentials in the cell bodies and axons. In other studies from this laboratory the synaptic potentials reported here have been shown to be capable of summation, so that transmission becomes possible through synapses which were otherwise blocked (Bishop *et al.* 1959*a*, *b*). Extracellular synaptic potentials can also be recorded from single geniculate cells (Freygang, 1958; Bishop, Burke & Davis, 1958) though it is clear that they must bear a rather indirect and complex relationship to the potentials recorded from populations of geniculate cells.

The geniculate synaptic potentials reported here are very similar to the so-called 'focal synaptic potentials' recorded with extracellular micro-electrodes from motoneurone pools in the spinal cord (Renshaw, 1946; Brooks & Eccles, 1947; Lloyd & McIntyre, 1949; Brooks, Downman & Eccles, 1950*a*, *b*). The spatial distribution of the current flow (Eccles, Fatt, Landgren & Winsbury, 1954) in relation to the dendrites, cell bodies and axons of the motoneurones also resembles that found in the lateral geniculate nucleus. The motoneurone focal synaptic potential has a duration of about 10·5 msec (Fig. 9 of Brooks & Eccles, 1947) and a late after-positivity of up to 50 msec was noted by Brooks *et al.* (1950*b*). The late after-positivity is much more clearly seen in the ventral-root recording after electrotonic spread along the intramedullary motor axons. With ventral-root recording maximum positivity is reached at 40 msec and the base line is regained at 100 msec (Brooks *et al.* 1950*b*). It is to be expected that the time course of the early phases of the ventral root recordings will be distorted by passive membrane electrotonus (Brooks & Eccles, 1947), though the late positive phase should be relatively unaffected. It is rather surprising that Frank & Fuortes (1955) did not find any evidence of these slow potentials in their intracellular records from intramedullary motor axons (cf. also Coombs, Curtis & Eccles, 1957). Similar focal and ventral-root synaptic potentials have been recorded from the frog's spinal cord (Brookhart & Fadiga, 1960).

The focal synaptic potentials can be clearly correlated with the intracellularly recorded synaptic potentials (Brock, Coombs & Eccles, 1952; Fatt, 1957*b*). The intracellular excitatory synaptic potential, however, reaches its summit more slowly and has a longer time of decay. Interest has centred around the time constant of decay of the intracellular excitatory synaptic potential and no systematic measurements seem to have

been made of the duration of the phase of depolarization. Published records, indicating a value between 15 and 20 msec, are very variable, presumably reflecting differences in the geometrical distribution of the synaptic depolarizations with respect to the locus of the micro-electrode and the distorting effect of the cable properties of the dendrites (cf. Frank & Fuortes, 1956; Fatt, 1957*b*; Rall, 1959). The initial depolarization is followed by a long-lasting hyperpolarization (Brock *et al.* 1952; Coombs, Eccles & Fatt, 1955). Maximum hyperpolarization, in one instance, was reached at about 35 msec and the total duration was about 100 msec, both values being similar to the positivity of the ventral root recordings. No later fluctuations of membrane potential appear to occur in the motoneurone. The phase of depolarization is clearly correlated with Lloyd's (1946) heterosynaptic facilitation curve and the late after-positivity can be correlated with a phase of depression both in respect to the amplitude of a testing focal synaptic potential and the level of reflex excitability (Brooks *et al.* 1950*b*). Coombs *et al.* (1955) suggest that the hyperpolarization phase of the synaptic potential is probably a true after-potential developing as an immediate consequence of the preceding depolarization, and therefore a partial activation of a process more intensively activated by the spike.

After-potentials

The after-potentials which follow the discharge of the geniculate cells resemble the synaptic potentials. The initial negative after-potential, lasting about 7·3 msec, is followed by an after-positivity which has a trough at 22 msec. The base line is crossed again at 80 msec, the late negative wave (peak at about 100 msec) leading on to further oscillations of potential that are again difficult to distinguish from background slow-wave activity. The orthodromic after-potentials recorded in the optic radiation are of reverse polarity to those recorded in the nucleus, and the antidromic after-potentials closely resemble those obtained at the same site by orthodromic stimulation (cf. Vastola, 1957, 1959; Bishop, Burke, Davis & Hayhow, 1958). Vastola (1957) reported that the negative portion of the post-synaptic response to orthodromic stimulation was 2–10 msec in duration. Later (1959) he gave values of 10–15 msec for the initial negative phase and 100–200 msec for the total duration of the after-positivity. Vastola also described the late negative or negative-going wave.

The focally recorded after-potentials in the motoneurone pools in the spinal cord following antidromic activation (Brooks *et al.* 1950*a*) have been studied, but the comparable after-potentials following orthodromic activation do not appear to have been investigated. The orthodromic after-potentials have, however, been described, using ventral root leads

(Brooks *et al.* 1950*b*). With intracellular recording both negative and positive after-potentials may be recorded following the spike discharge (Brock *et al.* 1952; Araki, Otani & Furukawa, 1953; Coombs *et al.* 1955). These after-potentials have nearly always been produced by antidromic activation of the motoneurone but the potentials are reported to be the same following both antidromically and orthodromically evoked spikes (Brock *et al.* 1952). Very little attention has been paid to the intracellularly recorded negative after-potential, but from published records it rarely seems to have a post-spike duration of more than about 2 msec. It rapidly decays to a large positive after-potential that reaches a maximum after 10–15 msec and has a duration of about 100 msec. By contrast the spike in the Renshaw cell may be followed by a negative after-potential lasting more than 40 msec (Eccles, Fatt & Koketsu, 1954). The after-potential mechanisms in the frog spinal motoneurones appear to be different from those in the cat (Machne, Fadiga & Brookhart, 1959).

With antidromic activation the components of the motoneurone spike may be readily separated (cf. Eccles, 1957) and these are found to have distinctive after-potentials. Although no detailed studies have yet been made it is clear that, taking the axon, initial segment and cell body in sequence, there is a progressive reduction in the duration of the negative after-potential, with finally a marked augmentation of the positive after-potential. Both the axonal and 'initial segment' spikes (IS spike of Coombs *et al.* 1957; A spike of Fuortes, Frank & Becker, 1957) are followed by a prominent negative after-potential, the positive after-potential being practically absent in each case. By contrast the 'soma-dendritic' spike (SD spike of Coombs *et al.* 1957; B spike of Fuortes *et al.* 1957) has a very brief negative after-potential and a deep and prolonged positive after-potential. The excitability of these components is in keeping with the pattern of their after-potentials (Coombs *et al.* 1957; Fuortes *et al.* 1957). The interpretation of motoneurone after-potentials is complicated by the generation of synaptic potentials by activation of recurrent motor-axon collaterals (Eccles, Fatt & Koketsu, 1954). The importance of this factor in the lateral geniculate nucleus is not clear (Vastola, 1959; Bishop & Davis, 1960). It is curious that the antidromic spike in the Betz cells in the motor cortex is not usually followed by any after-polarizations (Phillips, 1959).

Relationship between after-potentials and synaptic potentials

Comparing the geniculate synaptic potentials and the corresponding spike after-potentials (Table 3; Figs. 9, 10) the principal differences are the marked reduction in the duration of the initial negative phase when a spike is discharged and the marked augmentation of the amplitude of

the succeeding positivity (Figs. 9, *b*, *c* and 10). The duration of both the positivity and the subsequent oscillations of potential remain practically unchanged. A strikingly similar change can be seen to occur in the ventral-root synaptic potentials when a motoneurone discharge takes place (Brooks *et al.* 1950*b*). This change is to be expected in view of the pattern of the after-potentials that follow the cell-body spike (see above). The after-positivity which follows the motoneurone excitatory synaptic potential has the same duration as the after-positivity which follows the spike,

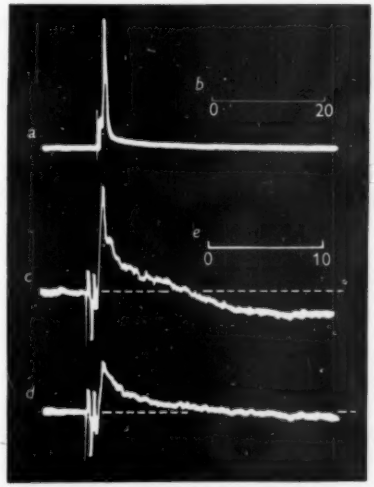


Fig. 9. *a*, Monophasic action potential and prolonged negative after-potential obtained from the optic nerve following antidromic activation of the contralateral optic tract. Time scale *b* (20 msec) refers to trace *a*. *c* and *d*, taken from Figs. 1 and 5, show the after-potential and LSD synaptic potential respectively, recorded at the same intrageniculate site following maximal shocks applied to the contralateral optic nerve. Time scale *e* (10 msec) refers to traces *c* and *d*.

i.e. 100 msec. Another factor has to be taken into consideration, namely the after-potentials of the post-synaptic axons. Assuming that the properties of the radiation axons resemble those of the optic nerve axons they will have a prolonged negative after-potential and practically no positive after-potential (Fig. 9*a*; Bishop, Jeremy & Lance, 1953; cf. Lloyd, 1951; Rudin & Eisenman, 1954; Coombs *et al.* 1957). This negativity in the axons will have the effect of reducing the amplitude and shortening the duration of the post-spike negativity recorded in the lateral geniculate nucleus.

Probably much more important than the axon, however, is the effect of potentials originating in the dendrites and the extent to which they survive the spike and thereby impose a continuing influence on the spike-

generating mechanism (Tasaki *et al.* 1954; Eyzaguirre & Kuffler, 1955*a*, *b*). The first component (IS or A) of an intercurrent antidromic spike is incapable of abolishing the motoneurone synaptic potential (Brock, Coombs & Eccles, 1953), presumably because it does not invade that region of the neurone where synaptic activity occurs. By contrast the second component (SD or B) largely removes the synaptic potential, because the spike now invades membrane involved in synaptic activity. The small residual potential that remains in the latter case has been variously interpreted as due to prolonged action of the synaptic transmitter (Curtis & Eccles, 1959) or to the fact that part of the dendritic surface does not have its synaptic potential wiped out by antidromic invasion, and that this residual synaptic potential then spreads electrotonically to the soma (Rall, 1959; cf. also Fatt, 1957*b*). Both factors may well be of importance in the one cell. The degree of antidromic invasion of the dendrites probably varies from one type of neurone to another and also in the one neurone under different conditions. Freygang (1958) has put forward the view that a large part of the soma-dendritic membrane of the geniculate neurone, though excited synaptically to produce a synaptic potential, is not invaded by a propagating spike. Even if the large dendrites are invaded it is likely that there will still be a significant spread of charge from uninvaded finer dendritic branches into the cell body and axon. In the crayfish isolated neurone (Eyzaguirre & Kuffler, 1955*a*) a persisting generator potential in the distal dendritic processes can lead to the initiation of impulses at a site along the axon at a distance of 0·5 mm from the cell body (Edwards & Ottoson, 1958). The long-continued depolarizing potential and repetitive discharge in the Renshaw cell have been attributed to the persistence of the transmitter substance as a result of the presence of diffusional barriers intimately related to the synaptic terminals on the cell (Eccles, Eccles & Fatt, 1956). Such a diffusional barrier might also lead to the accumulation of potassium released during activity and hence to the production of a negative after-potential such as occurs in the axon (cf. Narahashi & Yamasaki, 1960).

It is possible that the cell-body after-potentials of both motoneurones and geniculate neurones are, in fact, very similar, consisting of a very brief after-negativity and a deep and prolonged after-positivity without further oscillations of potential. The longer negative after-potential of the geniculate neurone and the later oscillations of potential would then be due to continuing spread of charge from the dendrites. One can regard the properties of geniculate neurones as being intermediate between those of the Renshaw cells on the one hand and of the motoneurones on the other. Part of the brief negative after-potential that follows the motoneurone SD spike may also be due to residual dendritic activity.

Relationship between after-potentials and recovery of responsiveness

The extent to which the after-potentials determine the recovery of responsiveness of geniculate synapses (Bishop & Evans, 1956; Bishop & Davis, 1960) following their discharge may now be briefly discussed. The curve of recovery of responsiveness of the r_1 spike is shown as the continuous line in Fig. 10, the ordinates representing the degree of supernormality and subnormality as a percentage of the unconditioned r_1 spike (for details see Bishop & Davis, 1960). The two early phases of supernormality have

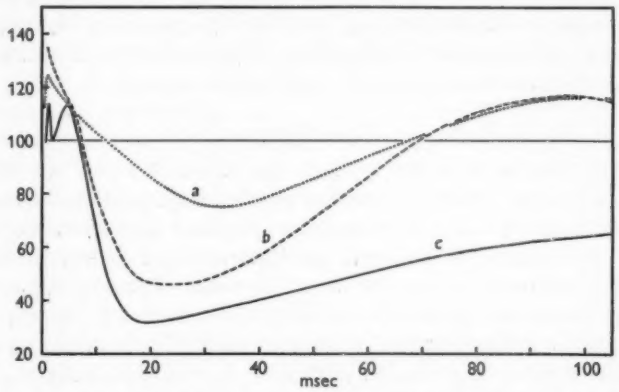


Fig. 10. *a* and *b*, mean time courses of the synaptic potentials and the after-potentials of geniculate neurones respectively; ordinates in arbitrary units. *c*, mean curve of recovery of responsiveness of geniculate synapses following a maximal conditioning shock applied to the optic nerve expressed as a percentage of the unconditioned test response (from Bishop & Davis, 1960).

a total duration of 6·7 msec. The following phase of depression is maximal at 19 msec and the recovery, which is still continuing at 100 msec, is not actually complete until about 2 sec. The geniculate after-potentials are shown as the broken line in Fig. 10, the ordinates being in arbitrary units. The negative after-potential has a duration of 7·3 msec and the positive after-potential has a trough at 22 msec. Thereafter the curve of recovery of responsiveness soon diverges widely from the after-potential pattern. The close correspondence that obtains, however, during the first 30–40 msec indicates that the after-potentials probably play a dominant part in determing the excitability of the neurone during this interval. Bishop & Davis (1960) considered the two early phases of supernormality as though they were of largely separate origin. It is more likely, however, that they represent two phases of the same process determined by the summed effect of the negative after-potentials of the cell body and axon and the residual

soma-dendritic synaptic activity. The brief intervening phase of depression or relative depression is possibly due to the brief interval of reduced or reversed current flow between cell body and axon that occurs when the radiation axons are occupied by the spike process.

The later stages of the recovery cycle are probably largely determined by presynaptic changes. An indication of the importance of presynaptic changes is the fact that, with heterosynaptic testing, the prolonged phase of depression of geniculate synapses lasts only 350 msec in contrast to the depression following homosynaptic testing which lasts about 2 sec (Bishop, Burke, Davis & Hayhow, 1958). Brooks *et al.* (1950*b*) have produced similar evidence which indicates that the late phase of the depression (i.e. after about 150 msec) in the recovery cycle of motoneurones following orthodromic activation is due to a presynaptic change.

Slow rhythms

It is becoming increasingly evident that the slow waves recorded from the cerebral cortex depend upon the rhythmic fluctuations of the membrane potential of the apical dendrites, whether the waves are spontaneously occurring or evoked (cf. particularly Clare & Bishop, 1955; Li *et al.* 1956) and the homology has been suggested of these potential waves to the post-synaptic potentials that may be recorded in the spinal cord and lateral geniculate nucleus (cf. Bremer, 1958). The results of the present study also indicate that the dendrites of the principal cells may be responsible for the slow rhythms that are recorded in the lateral geniculate nucleus. Moreover, the principal cells are almost certainly the only neurones present in the nucleus whose morphological arrangement would permit of the generation of the recorded potential fields. The geniculate rhythms continue even though the afferent input from the retina has been removed. The rhythms do not therefore depend upon the spontaneous discharge of retinal receptors. Whether the dendrites of the geniculate neurones are actually capable of continuous self-excitation or not, it is clear that, under normal circumstances, they must be conceived as being at least only just below this self-excitatory level, because they may be triggered into prolonged rhythmic activity by synaptic potentials that are well below the level necessary to initiate a propagated discharge. This occurs when the synapses on the principal geniculate neurones are blocked by large doses of LSD. If one accepts the view that LSD acts by competitive antagonism to the normal transmitter it is likely that all the excitatory synapses on the principal cells will be blocked by its action. This places a clear restriction on the mechanism used to explain the neuronal synchronization that must occur in the production of these rhythmical potentials. One explanation of neuronal synergy (cf. Bremer, 1958) involves

intricate synaptic connexions between the active cells by short axon interneurones and by recurrent collaterals of long axons. Although LSD may be without effect on the short axon interneuronal circuits in the lateral geniculate nucleus, it is unlikely that the principal neurones themselves can be included in the recurrent loops but must be regarded as non-propagating off-shoots.

Prolonged rhythmic waves, indistinguishable from those arising spontaneously, may be triggered off both orthodromically by synaptic potentials and antidromically by optic radiation volleys. The first cycle of the triggered activity, however, is different from the later slow waves. The first cycle always commences with a relatively brief negative phase followed by a positive phase which has a greater amplitude and is more prolonged than those that succeed it (Figs. 8, 10). The general conclusion suggested as a result of this study is that the geniculate neurones are capable of generating three kinds of non-propagating potentials, namely: (1) slow rhythmic oscillations due to fairly prolonged or possibly sustained self-excitation; (2) synaptic potentials due to orthodromic volleys, having an initial negative phase, lasting somewhat longer than 12 msec, followed by a prolonged after-positivity; and (3) after-potentials that arise as a result of the generation of a cell-body spike, having a very brief initial negativity (less than 7 msec), followed by a deep and prolonged after-positivity. It is not possible to assign these potentials to either dendrites or cell-body with any certainty. The possible restriction of the spike to the cell body would restrict the after-potentials to the cell body also. The other two potentials possibly involve both structures with the prolonged rhythmic activity being largely of dendritic origin. It is likely that the mechanisms responsible for all these potentials may operate concurrently so that the potentials actually recorded result from their complex interaction.

SUMMARY

1. The lateral geniculate neurones have been activated, orthodromically and antidromically respectively, by means of electrical stimulation of the optic nerve in the orbit and the optic radiation terminals in the cerebral cortex of the cat. The geniculate responses have been recorded extracellularly by means of a micro-electrode inserted down through the intact cerebral cortex.

2. Measured from the onset of post-synaptic activity, the initial negative after-potential of the orthodromic response has a mean duration of 7·3 msec; the subsequent positive after-potential has a sharp decline to a maximum at about 22 msec and returns to the base line at about 80 msec. There is a late negative peak soon after 100 msec. The subsequent oscil-

lations of potential, which have a mean frequency of 9 c/s, cannot be distinguished from the spontaneous slow rhythms.

3. The orthodromic after-potentials increase in amplitude with increasing afferent stimulation but are not significantly altered in time course. Recorded in the optic radiation the after-potentials have a similar time course but are of opposite polarity to those recorded in the nucleus. By using iron deposition to label the recording site the level at which the potentials reversed sign was found to be the upper surface of the nucleus.

4. Except for the initial after-negativity the antidromic after-potentials are the same as those evoked at the same site by orthodromic volleys.

5. Synchronous repetitive firing of geniculate neurones was frequently associated with the negative after-potential and occasionally unit repetitive firing was observed in relation to the negative peaks of the later slow rhythms. The repetitive firing ceased with the onset of the positive after-potential.

6. Synaptic potentials were recorded while the geniculate synapses were blocked by lysergic acid diethylamide and as a result of post-tetanic delayed depression, the level of block being monitored by recording from the cerebral cortex.

7. The time course of the synaptic potentials was the same in either case. Measured from the onset of post-synaptic activity the initial negativity has a duration of about 12 msec, the succeeding positivity reaches its maximum at about 25 msec, the base line is recrossed soon after 60 msec and the late negative wave has a peak at about 100 msec. The subsequent oscillations of potential are indistinguishable from the spontaneous slow rhythms which also occur in the nucleus during synaptic block.

8. In the temporary absence of spontaneous rhythmic activity long-continued oscillations of potential may be triggered off both by orthodromic and antidromic volleys.

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SOME FACTORS AFFECTING MILK SECRETION BY THE ISOLATED PERFUSED MAMMARY GLAND

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In recent years the perfused mammary gland has been used by a number of workers in the study of milk secretion. The method is open to criticism because both the blood flow and metabolic rate may be much lower than normal (Linzell, 1954) and in the cow the incorporation of radioactive phosphorus very low (Barry, 1952). In contrast, the yields of milk obtained by Tindal (1957) in 3–4 hr perfusions of cows' udders were surprisingly good (41 % of normal in the first 2 hr). This suggests that much of the milk obtained may have been preformed.

Similar results in all respects were obtained by J. M. Barry & J. L. Linzell (unpublished) in perfused goats' udders, which are technically easier to work with. We felt that a more detailed study of this preparation would indicate its reliability and limitations, and so help to assess the usefulness of mammary perfusion for biochemical studies.

In the work to be described we have tried to decide whether milk can be secreted in isolated perfused glands, using three criteria:

(*a*) the quantity and composition of the milk in relation to that produced by the glands in the conscious animal before perfusion;

(*b*) the responses to variations in the availability of known milk precursors produced by alterations in perfusate flow and composition; and

(*c*) the transfer of labelled substrates from blood to milk on the assumption that, given a steady blood concentration of a radioactive atom, a derived milk product should eventually have the same specific activity.

Preliminary communications have been given to the Physiological Society (Cargill-Thompson, Drury, Hardwick, Linzell & Tucker, 1958), and to the Biochemical Society (Hardwick, Linzell & Price, 1960).

METHODS

The detailed procedure and apparatus have been gradually evolved during the experiments. The majority of alterations to the apparatus were introduced for convenience, speed of removing the glands, and ease of assembling and cleaning the apparatus, and are not thought to have changed the method materially.

Apparatus. Initially the perfusion circuit was a simple arrangement in series of reservoir–pump–mammary gland–flow recorder–oxygenator–reservoir. To maintain thorough mixing of the substrates being run into the perfusate, and to provide extra oxygenation for large glands, an additional circuit of pump–oxygenator–reservoir was added later. The final arrangement was reservoir *A*–pump *A*–mammary gland–flow recorder–oxygenator *A*–reservoir *B*–pump *B*–oxygenator *B*–reservoir *A*. The reservoirs were joined by a narrow tube, and substrates were added to reservoir *B*. The reservoirs and pumps were situated n a water-bath, with the rest just above. The whole apparatus was enclosed in a plastic box resting on the rim of the water-bath, which therefore partly controlled the temperature and humidity of the air in the chamber. The pumps were of 7–12 ml. capacity (see Linzell, 1959*a*) driven by Dale–Schuster pumps below the reservoir. Apart from the walls of the oxygenator (Hooker, 1915, type), which were of glass, the circuit consisted mainly of Perspex and silicone rubber. Some preliminary experiments were carried out with the pump set to deliver constant-volume inflow, by recording perfusion pressure and adjusting the Dale–Schuster pump to maintain the pressure at physiological levels. The majority of perfusions, however, were performed at constant pressure with a Starling by-pass (the overflow blood going to oxygenator *B*). Venous outflow was measured with a modified Gaddum (1929) recorder. The total capacity of the circuit was about 1 l. and usually contained about 700 ml. of perfusate.

Animals

In fifty experiments the majority of animals were of mixed breed; thirteen were pure-bred Saanens and one was Anglo-Nubian. Age and stage of lactation and the weight of the glands (200–1300 g) made no apparent difference to the success of perfusion. Castrated male Saanen goats were kept as blood donors.

Perfusate

In the majority of experiments the perfusate consisted of 18–35 %, average 26 % (v/v), washed goat red cells (normal haematocrit 23–35 %), suspended in Krebs's solution containing 7 % goat albumin, heparin (15 u./ml.) with penicillin and streptomycin (each, 100 u./ml.). In some experiments glucose (100 mg/100 ml.) and Na acetate (10 mg/100 ml.), or some of the substrate mixture under test, were added. The red cells were mixed with the albumin-Krebs's solution just before the experiment, filtered through a 5 μ sintered stainless-steel filter (Adelphi Manufacturing Co., London, N. 1) and circulated through the apparatus to equilibrate with air or oxygen plus 7 % CO_2 for 30 min at 33° C before the gland was removed. The use of this filter removed small particles which had in previous experiments caused emboli, and obviated the necessity of including filters in the circuit during perfusion. Throughout the paper the perfusate flow is referred to as 'blood' flow and the supernatant fluid as 'plasma'.

The detailed preparation of this perfusate, the reasons for using it, and its comparison with autologous whole blood, are as follows:

Saline. This was the Ringer-bicarbonate solution of Krebs & Henseleit (1932). In recent experiments the Na has been increased to 153 m-equiv/l. and the K reduced to 4·2 m-equiv/l., in accordance with analyses of goat plasma (Linzell, 1960*b*). In a preliminary experiment with dextran and red cells, a gland perfused with Krebs–Henseleit medium had a slightly better 'blood' flow and developed less oedema and fewer petechial haemorrhages than the opposite gland perfused with Ringer's solution.

Albumin. This was prepared aseptically from mixed goat plasma by the method of Kekwick & Mackay (1954). Electrophoretic analysis by Dr A. E. Pierce of this Institute showed that one preparation consisted of 77 % albumin, 12 % α, 2 % β and 9 % γ globulin, and was not significantly different after a 6 hr perfusion. In four experiments goat albumin gave better 'blood' flows and less final oedema and haemorrhage than dextran (2) or goat plasma (2). During a perfusion with goat albumin, 60 min after the addition of an equal volume of 6 % dextran in Krebs's solution to one gland, blood appeared in the lymph. Bovine

albumin gave poorer flows than dextran, and moreover when injected intra-arterially into glands perfused with goat albumin produced a slight vaso-constriction. Dialysed goat plasma was more suitable but, in spite of the addition of heparin, tended to form fibrin threads in parts of the apparatus after some hours of perfusion. Additional advantages of albumin over dextran are that it slowly inactivates 5-hydroxytryptamine (5-HT), has some buffering power and binds Ca, fat and cortisol.

Red cells. These were prepared from blood drawn aseptically from castrated male goats the day before the experiment. The blood was centrifuged at 1340 *g* for 45 min and the plasma and the top third of the cells discarded. This process was repeated after suspending the bottom two-thirds of the cells in 1% NaCl and the remaining bottom layer of cells was washed twice in 1% NaCl, stored during the night, and given a final wash in Krebs's solution on the morning of the experiment.

This perfusate was used instead of blood because of the very high 5-HT content of goat blood platelets (Cargill-Thompson, Hardwick & Wiseman, 1957), which do not easily break down so that 5-HT could not be removed by dialysis. 5-HT is vasoconstrictor in goat mammary glands and the importance of removing it was shown in one experiment (Fig. 1) and confirmed subsequently. In thirteen perfusions with washed goat red cells suspended in goat albumin-Krebs's solution, the maximum blood flows were 51 ± 19 (S.D.) ml./100 g/min (range 20–81) and the mean flows in experiments lasting 3–8 hr were 38 ± 19 ml./100 g/min (range 10–70), associated with 'plasma' 5-HT levels of less than 0·2 μg/ml. (Unless otherwise stated all measures of variability are standard deviations.) These compare favourably with the flows in conscious goats of 45 ± 7 ml./100 g/min (Linzell, 1960*a*), and are in marked contrast with the maximum flows obtained with autologous whole blood in two perfusions, of 9 and 16·5 ml./100 g/min in the goat and of 4 and 10 ml./100 g/min in the cow (Tindal, 1957). Although maximum flows of 29–60 ml./100 g/min have been obtained by incorporating autologous lungs in the perfusion circuit, the presence of another tissue would make the interpretation of isotope experiments more difficult.

The 5-HT inhibitors available when this work was started all caused some vasoconstriction in goat mammary glands perfused *in situ*, and the addition of dihydroergotamine or lysergic acid diethylamide (LSD) 1 μg/ml. to the blood in two perfusions produced maximum blood flows of only 8 and mean flows of 7 ml./100 g/min. Similarly Tindal (1957) obtained maximum flows of only 15 ± 6 ml./100 g/min in the cow's udder, using dibenamine or dibenzyline. More recently we have obtained better results with autologous blood by using bromolysergic acid diethylamide (BOL 148, Sandoz) 0·6 μg/ml. In three experiments (with substrates) the maximum flows were 27·5, 34 and 33 ml./100 g/min and the mean flows in perfusions lasting 18, 13 and 14 hr were 22, 19 and 20 ml./100 g/min. In each of these experiments the opposite gland was perfused with the usual perfusate plus substrates and no significant difference in milk yield was observed (Fig. 5).

pH of perfusate. The pH (at the temperature of perfusion) of the initial perfusate varied from 7·2 to 7·5, and during perfusions the range was from 6·8 to 8·0. There was no apparent correlation between the pH and the mean blood flow or the total milk yield. There was a general tendency for the pH to rise during perfusion, an effect we believe to be due chiefly to the accumulation of Na^+ as the glands used up the acetate from sodium acetate. We tried ammonium acetate instead, but the NH_4^+ also accumulated to levels we felt might be toxic (90 mg/100 ml.). The addition of small amounts of lactic or acetic acid to reduce a rising pH did not apparently improve the milk yield or blood flow, and caused haemolysis.

Sterility. Although no attempt is usually made in physiological experiments to prevent bacterial growth, it was felt desirable to reduce this to a minimum, especially in experiments lasting up to 24 hr. The perfusate was prepared aseptically but penicilllin and streptomycin were included because the apparatus was clean but not sterile and the tissue was removed cleanly but not aseptically. Broth cultures made from the final perfusate showed slight growth at 37° C after 2–4 days in most experiments, but usually no growth at room tem-

perature. In a few cases the final perfusate showed no growth at all, but these experiments were not noticeably more successful, and there was no correlation between the amount of growth or the bacterial count and the amount of milk secreted in any of the experiments.

Incompatibility. The use of red cells and albumin from donor animals raises the possibility of incompatibility between these and the mammary tissue. Routine cross-matching tests (Drury & Tucker, 1958) capable of detecting the presence of very weak antibodies were carried out between the donor red cells, and albumin and the recipient red cells and serum by Miss E. M. Tucker, of this Institute. In a few cases weak haemolysins were demonstrated in the recipient's serum which reacted with the donor red cells, but this apparently had no adverse effect on the perfusion.

To test the importance of compatibility more decisively we immunized a recipient goat with the whole blood of two donors before the experiment, and obtained a high titre of haemolysing antibody to the red cells of both donors. Although at the time of the experiment the recipient animal showed marked reactions to an intravenous injection of a few

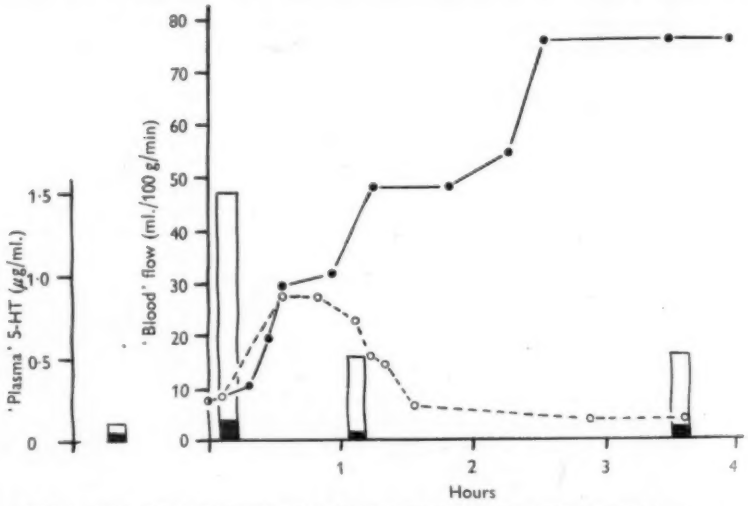

Fig. 1. 'Blood' flow through 2 glands of a goat which were perfused with dextran-Krebs's solution. One gland had washed goat red cells (■●) and the other the same red cells plus white cells and platelets (○ □) added to the perfusate (19 % by volume). Equilibration of the perfusate before perfusion released 5-HT.

millilitres of mixed donor whole blood (fall in B.P. and apnoea), perfusion of the glands with red cells and albumin prepared from the blood of the two donors was successful (Fig. 5*b*). Recipient blood was washed out of the tissue as usual before perfusion and, even though antibody appeared in the lymph and milk throughout perfusion, there was no evidence that it appeared in the perfusate because the donor cells were not sensitized. As the natural antibodies are very weak compared with the immune antibodies, it can safely be inferred that when red cells in albumin are used as perfusate, incompatibility will be of little significance.

Substrates

In preliminary experiments on the basic perfusate, a solution of glucose 5·5 % and Na acetate 1·37 % was run in at 9 ml./hr. In most experiments the substrate solution had the

following composition (g/l.): glucose 75, acetic acid 25, Na-β-OH-butyrate 4, $Na_2HPO_4.2H_2O$ 2; and the following L-amino acids: alanine 0·4, arginine 0·8, asparagine 0·8, aspartic acid 0·4, cysteine 0·4, glutamine 2, glutamic acid 2, glycine 0·5, histidine 0·5, hydroxyproline 0·4, isoleucine 1, leucine 2·5, lysine dihydrochloride 1·35, methionine 0·7, phenylalanine 1·6, proline 1·9, serine 1·25, threonine 0·8, tryptophan 0·25, tyrosine 0·2, valine 1·7, and goat albumin 30. The pH was adjusted to 6·0–6·5 with about 40 ml. 10 N-NaOH/l. This mixture was calculated so that 1 ml. of solution provided all the precursors (including water) for the formation of 0·5 ml. of goats' milk. It was run into the perfusate continuously at 5–30 ml./hr. The amino-acid pattern was that of casein, except that tyrosine, being sparingly soluble, was largely replaced by phenylalanine, and that cysteine and hydroxyproline were increased. $CaCl_2$ (1·1 g/l.) was added at a rate found by experience to keep the perfusate Ca level approximately constant (about 8 mI./hr).

Unfortunately the inflow of the more complex substrate solution sometimes caused a fall in blood flow. Maximum flows in experiments where substrates were used were 33 ± 18 ml./100 g/min and mean flows only 20 ± 12. The intra-arterial injection of acetate and lactate in these perfusions and into the glands perfused *in situ* with the animal's own blood sometimes caused vasoconstriction, although in other experiments they produced vasodilatation, as did also hypertonic glucose and sucrose. However, the apparatus was modified to make sure that the substrates were thoroughly mixed with the perfusate before reaching the gland.

Operations

Anaesthesia. We have preferred to remove the glands under anaesthesia rather than after killing the animal, because the long preparatory dissection could then be done carefully with the blood supply intact. In the majority of experiments the glands were removed under epidural anaesthesia. This was induced, 1 hr after a tranquillizing dose of chlorpromazine (1·50–1·7 mg/kg), with 2% lignocaine + adrenaline: usually 5–7 ml. was injected into the lumbosacral space and an additional 3 ml. on each side between L1 and 2.

In preliminary experiments general anaesthesia was induced with pentobarbitone (10–20 mg/kg) intravenously and maintained with cyclopropane and O_2 in a closed circuit. When, in such perfusions, we infused amino acids, fat and 5% plasma in addition to the small quantities of glucose and acetate used, there was no increase in milk yield as compared with the opposite gland receiving glucose and acetate only. We thought this might be due to the use of general anaesthesia, because in thirty recovery operations on goats under this anaesthesia (including udder operations) the mean milk yield 5–8 hr after induction was reduced by 65% (range from 28% increase to 100% decrease) as compared with the comparable period on the 3 previous days. In contrast to this, in eleven similar recovery operations done under epidural anaesthesia plus chlorpromazine, the average milk yield was not affected (range from 20% increase to 22% decrease).

However, we have recently perfused the glands of three goats removed under general anaesthesia with adequate amounts of all substrates and obtained milk yields similar to those of glands removed under epidural anaesthesia, which suggests that in our earlier experiments failure was not due to the general anaesthetic but to insufficient acetate and glucose (Fig. 6). We still prefer epidural anaesthesia because the milk yield during removal was reduced by 50% on average under general anaesthesia but only by 15% under epidural (Table 1).

Surgical procedure. After sterilizing the skin the two glands forming the goat's udder were separated, removed and perfused independently, so that one could be compared with the other. The glands were cooled with ice packs and dissected free one at a time leaving the main vessels intact to maintain the circulation until just before removal. Local anaesthetic (0·5% lignocaine) was injected around the nerves before dissection. The main external pudic and usually the lymphatic vessels were cannulated and all other vessels tied off or cauterized with electrocautery. The blood left in the gland was discarded either by heparinizing the

animal before removal and rejecting the first 50 ml. perfused through the gland (under general anaesthesia) or by washing through with 10–50 ml. heparin-Krebs solution just after removal (under epidural anaesthesia). Each gland was suspended in the apparatus in a natural position by the lateral skin and median suspensory ligament, over a funnel to allow the small amounts of blood or perfusate oozing from the cut surfaces to be caught and returned to the reservoir through a fine cotton filter.

Milking

Before the experiment the animals were milked twice daily by hand at 9.30 a.m. and 5 p.m. and the yield of each gland was recorded separately. Since milk secretion is a steady continuous process (Linzell, 1959*b*), the average hourly yield was calculated from the mean daily yield for 7 days before the experiment (except in a few cases where it increased or decreased significantly during this period, when a shorter period was used.) The validity of this procedure was also checked by milking conscious goats hourly, after I.V. oxytocin 200 m-u. Milk samples for 3 days before the experiment were bulked for analysis to obtain the mean composition before perfusion.

On the morning of the experiment the goat was milked out completely, with 200 m-u. oxytocin, before anaesthesia, so that the residual milk obtained at the start of perfusion (0·75–2·50 hr later) gave the secretion rate and composition of the milk being secreted under the operation, immediately before perfusion.

In order to obtain the milk for measurement and analysis, 40 or 100 m-u. oxytocin was injected intra-arterially at the start of perfusion and every hour thereafter. The lower dose was used recently because vasoconstriction occurred in some glands. Responses to oxytocin have been obtained for up to 27 hr of perfusion and on no occasion was any more milk obtained by giving a second equal or larger dose immediately afterwards. In the first experiments the teats were cannulated, but, since it was still found necessary to massage the glands to get all the milk out, cannulation was abandoned and the glands were milked in the normal way.

Period without blood flow

The period between cutting off the normal blood supply and starting the perfusion was recorded in each experiment. In twenty-six experiments under epidural anaesthesia, this was 2–9 min (mean, 4) but in sixteen others, where it averaged 20 min (13–27), no significant difference in the milk yield or blood flow during perfusion was detected. In spite of these findings we have tried to keep this period as short as possible.

Temperature of perfusion

The effect of temperature was tested in two experiments. In each case one gland perfused at 39° C (the venous blood temperature of conscious goats) gave less milk for a shorter period than the opposite gland perfused at 32° C. In the majority of experiments, after cooling the glands during dissection, the initial temperature of the perfusate was set at 30° C and gradually increased to a maximum of 34–36° C. Statistical analysis of these experiments, and of an earlier group perfused at 38–39° C throughout, failed to show any significant difference in milk yield.

Oxygen tension

In some experiments the glands were very sensitive to a raised oxygen tension, showing a marked fall in blood flow that occurred in response to changing from air + 7 % CO_2 to O_2 + 7 % CO_2. This was not seen in all cases, and was most marked in the early stages. It was not always possible to use air throughout in the case of large glands using a lot of O_2. In these cases some O_2 was added cautiously to raise the O_2 content of the arterial blood. 7 % CO_2 was used to keep the plasma CO_2 of the perfusate at about the normal level for lactating goats.

General course of perfusion

The perfusions were carried on for 5–27 hr (average 12). Generally the artery became constricted during cannulation and the initial 'blood' flow was low (about 10 ml./100 g/min), rising during the first few hours of perfusion (Figs. 1, 2, 4, 6, 7). Thereafter, in most experiments in which substrates were infused, the pattern was variable, unpredictable and often uncontrollable. As the work proceeded it became clear that the amount of milk secreted was related to the blood flow (see Results), so we have paid great attention to this factor. We have already mentioned that a reduced or variable blood flow has been associated with solid emboli, some substrate constituents, oxytocin and a high O_2 tension and in recent experiments these have been controlled. We believe also that micro-bubbles have reduced the blood flow in some experiments. Albumin froths easily and the foam is very stable, so that careless injections into the reservoirs tend to produce micro-bubbles. This trouble has recently been eliminated by great care and larger bubble traps.

Our experience has been that, unless a fall in blood flow is arrested quickly, it tends to continue and cannot then always be reversed. Therefore, in recent experiments we have promptly injected 40–100 mg of adenosine intra-arterially as soon as the flow started falling (see Fig. 7) and in addition have placed papaverine 2–5 % on the vessels during cannulation and at the start of perfusion. Neither of these substances has affected the milk yield and with these modifications in technique fully normal flows have been obtained (see Results with a modified technique, p. 565). An important finding was that, in the majority of cases when the blood flow was low, the terminal intra-arterial injection of Indian ink revealed a patchy distribution in the tissue, showing that in such cases large areas of the glands were not being perfused.

Lymph flow has also been very variable (Fig. 7), ranging from zero to normal figures (Linzell, 1960*b*). Zero flows were obtained only when cannulation had been carried out before heparinizing the glands. As might be expected, lymph composition reflected that of the perfusate in respect of Na, K, Ca, P, acetate, glucose and protein. In most experiments perfusion could be prolonged for some hours after the milk yield fell below 5 % of the previous hourly yield, and an uptake of O_2 and substrates continued. Oedema, as judged by 20 % or more increase in weight at the end of perfusion, occurred in about half the experiments. However, this was very rarely marked before milk secretion had ceased and often occurred in less than an hour as a terminal event. Other terminal pathological features observed occasionally were petechial haemorrhages, blood in the milk and lymph and congestion of the tissue.

Histologically the secretory cells appeared normal at the end of the experiment except that, in some cases, fat was almost entirely absent from the secretory cells. Haemolysis was detected in the centrifuged 'plasma' after about 4 hr and increased steadily thereafter. The haematocrit tended to fall owing to the addition of substrates, unless there was marked oedema. The addition of substrates also raised the tonicity of the perfusate towards the end of perfusion.

Analytical methods

Perfusate. 'Blood' gases were measured in the manometric apparatus of Van Slyke; 'blood' sugar by the method of Somogyi (1952) with Nelson's (1944) precipitant; 'blood' volatile fatty acids by steam distillation (Annison, 1954); Ca and Mg by EDTA titration of the oxalic and ammonium phosphate salts (Wilson, 1955); amino acids by the procedure of Hamilton & Van Slyke (1943); inorganic phosphate was estimated by the method of Fiske & Subbarow (1925) and later by that of Berenblum & Chain (1938).

Milk. Lactose was estimated by Somogyi's (1952) method after phosphotungstic acid–Zn acetate precipitation (Grimbleby, 1956). Fat, total solids and ash in macrosamples by British Standard 1741 (1951) and in microsamples (1·5–2 ml.) by drying at 95° C on paper, extracting

the fat by Soxhlet and ashing at 500° C. Ca was determined in ash by dissolving in N-HCl, neutralizing, precipitating as the oxalate and titrating with EDTA (G. Bull, unpublished).

In much of the work casein was precipitated at pH 4·6, washed twice, digested in sulphuric acid and the nitrogen determined by Kjeldahl. Albumins and globulins were estimated by Moir's method (Davis & Macdonald, 1953). Recently, because these fractions were found by Dr A. E. Pierce to be electrophoretically complex, a modification of Rowland's (1938) method was used. Total N, non-casein N (casein precipitated at pH 4·6) and non-protein N (protein precipitated by 15 % trichloroacetic acid) were determined and casein and non-casein protein obtained by difference. Inorganic phosphate was determined in trichloroacetic acid filtrates and casein P in sulphuric acid digests by the method of Fiske & Subbarow (1925) or of Berenblum & Chain (1938). The N:P ratio of casein was 19–22 on a weight basis, which is close to the accepted figure for bovine casein.

When we used ^{32}P, the activities of the reduced phosphomolybdate extracts of plasma, milk and casein were determined in a liquid counter and the specific activity expressed as counts/min/μg P. Although casein was not defatted subsequent tests indicated that the likely error due to the presence of phospholipid P in determinations of casein specific activity was of the order of 10 % or less. In the conscious goat, we found that milk phospholipid is labelled much more slowly than casein and thus contamination with phospholipid would reduce casein values.

RESULTS

The majority of the results were obtained with the technique described in the Methods. We give the results of recent experiments separately because the technique has been modified in the light of our findings and the results have been better.

In most experiments the milk yield generally increased or remained constant during the first 3–4 hr, and thereafter declined. This pattern of secretion raises the problem of comparing experiments in which milk was secreted for different lengths of time. Although the hourly yield can be satisfactorily expressed as a percentage of previous yield, to do this for the total milk obtained in the experiment may be misleading, because experiments where milk was secreted for only a short time (4–6 hr) appear better than much longer ones. We have, therefore, expressed the total milk yield in 'hoursworth', which is the total yield divided by the average hourly yield during the 3–7 days before the perfusion. This represents the time previously required to secrete the volume of milk obtained during the perfusion. An hourly secretion rate of less than 5 % has been arbitrarily taken as zero.

Milk secreted without added substrates

Glands perfused entirely without substrates produced about 1·1 'hoursworth' of milk, of which 0·8 'hoursworth' was obtained in the first 2 hr, even though they were carefully milked out with oxytocin at the start of perfusion. For example, glands giving 20 ml./hr before the experiment gave on average 16 ml. in the first 2 hr (40 %/hr) and 6 ml. thereafter. After the 4th hr the yields were generally negligible (Fig. 2). These facts suggest

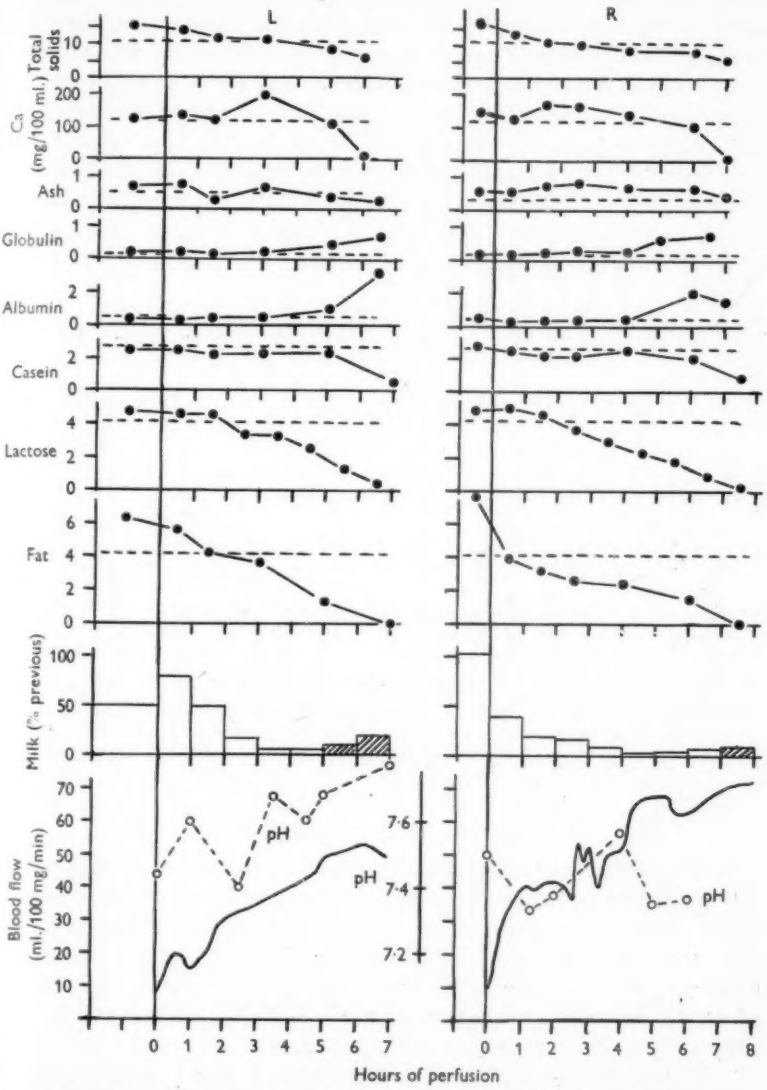

Fig. 2. Observations made on two glands (from one goat) perfused entirely without substrates, weighing 420 g (L) and 430 g (R) and yielding 28 and 31·5 ml./hr before perfusion. Marked oedema developed late in the experiments and the final secretion resembled tissue fluid (cross-hatched). Final weights 680 and 635 g. Glands removed under epidural anaesthesia (anoxic period 5 and 3 min) and perfusion started at 0 hr. Composition of milk secreted for 3 days before the experiment indicated by interrupted lines. Ordinate scale, except where marked, g/100 ml.

either that the milk obtained was preformed or that the tissue had considerable stores of available energy and milk precursors, or both. The composition of the first 2 hours' milk was not significantly different from that produced before the experiment. The very small quantity of milk obtained after 2 hr showed declining lactose and fat concentrations but the casein and ash remained normal until about the 6th hour (Fig. 2). In our preliminary experiments testing various perfusates we also regularly obtained about 1 'hoursworth' of milk in perfusions lasting 4–8 hr, when only small amounts of acetate and glucose but no amino acids were added.

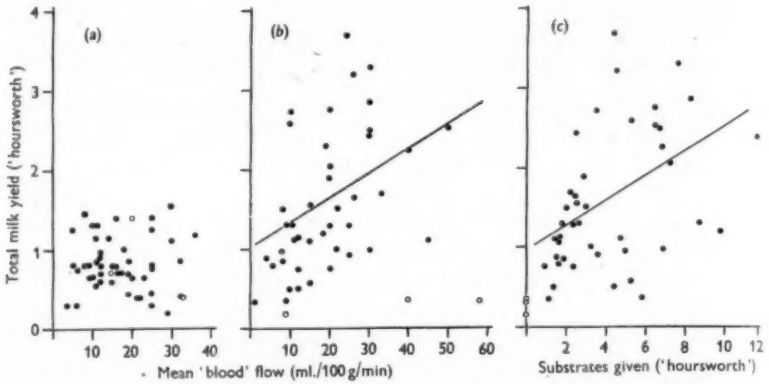

Fig. 3 (*a*, *b*). Relation between milk obtained during perfusion and the mean 'blood' flow (*a*) first 2 hr of perfusion; (*b*) after 2nd hour. (*c*) Relation between milk yield after the 2nd hr and the total substrates added. Glands given substrates ●; glands given nothing ○. The regression lines have been calculated from the data, allowing in (*b*) for the amount of substrates given and in (*c*) for the mean 'blood' flow.

Effect of adding substrates

Initially. Glands given substrates produced no more milk in the first 2 hr (0·8 'hoursworth') than those given none but they continued to secrete for longer (Figs. 3–7), so that the average yield after the first 2 hr was $1{\cdot}66 \pm 0{\cdot}87$ 'hoursworth'. In these experiments secretion continued to be above 5% of the normal yield for 12 ± 3 hr. The composition remained essentially normal for most of this period (Table 1). Lactose and fat concentration fell in some cases, generally terminally, but casein and ash did not. Indeed, proteins and total solids sometimes rose towards the end of perfusion, possibly in association with a hypertonicity of the perfusate at this stage due to large quantities of added substrates. The fat content varied more than the other components, but this was also seen when conscious goats were milked hourly after oxytocin. It is well known that the

fat concentration increases in successive portions of milk drawn off during a normal milking in ruminants and, unless oxytocin is injected, about 15 % of the milk is left behind, with a fat concentration of 2–3 times normal. This probably accounts for the high fat content of the milk removed at the start of perfusion (Figs. 2, 6, 7).

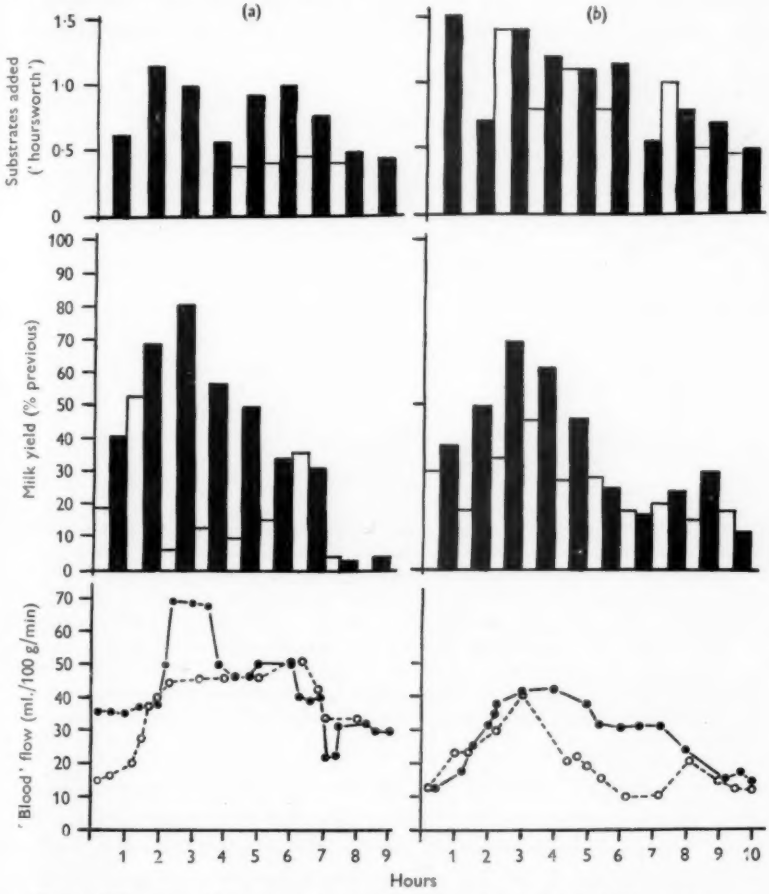

Fig. 4. Milk secreted in two experiments where one of each pair of glands was given substrates from the start (black) and the other starved initially (white). (*a*) Glands weighing 402, 434 g yielding 16 ml./hr before the perfusion. Final weights 438 and 435 g. Glands removed under epidural anaesthesia. (Anoxic periods 17 and 19 min.) (*b*) Glands weighing 661, 775 g yielding 10 and 13 ml./hr previously. Final weights 812 and 1080 g. Glands removed under epidural anaesthesia. (Anoxic periods 26 and 25 min.)

The milk yield after 2 hr varied considerably (from 3·88 to 0·35 'hours-worth'), and we have been able to correlate this with two factors. First, there was a strong positive correlation between the milk yield after 2 hr and the mean 'blood' flow during the same period ($P < 0{\cdot}05$), including experiments where the flow fell at an early stage from a known cause, such as embolism (Fig. 3*b*). Secondly, the milk yield was related to the total amount of substrates added throughout the perfusion (Fig. 3*c*)

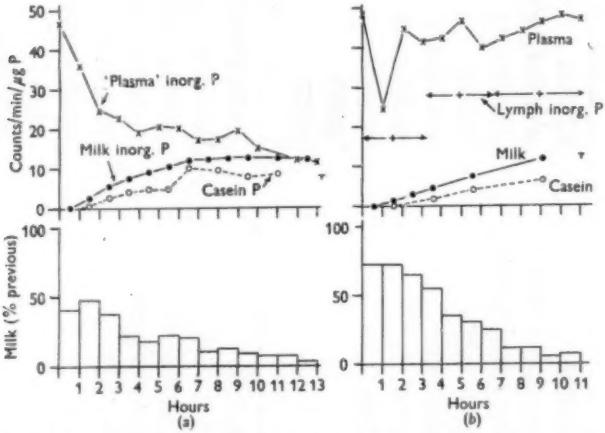

Fig. 5. The rate of incorporation of radioactive P from the 'plasma' (×) into milk inorganic P (●) and casein (○); the specific activity of inorganic P of tissue at end of perfusion (T); lymph (+). (*a*) Gland weighing 700 g yielding 36 ml./hr before the experiment perfused with the animal's own blood plus BOL 1 μg/ml. Removed under general anaesthesia (anoxic period 11 min). (*b*) Gland weighing 520 g yielding only 5 ml./hr before experiment perfused with albumin-Krebs's solution plus homologous red cells against which the animal had been immunized. The final specific activity of the milk and tissue of the opposite gland (previous yield 19 ml./hr) was 50% of the 'plasma' level. Glands removed under epidural anaesthesia (anoxic period 5 min).

expressed as the amount of milk (in 'hoursworth') which this quantity could theoretically make ($P < 0{\cdot}05$). This has been tested over a large range of amounts of substrate offered only for glands that previously had a fairly low yield (< 20 ml./hr). High-yielding glands were given relatively low amounts of substrate in most cases, but within these limits the relationship still held. Although it is unfortunate that in the preliminary work we could not always maintain fully normal 'blood' flows throughout perfusion and did not always add optimum amounts of substrates, nevertheless the apparent dependence of milk yield on these variables strengthens the belief that the glands were synthesizing under these conditions.

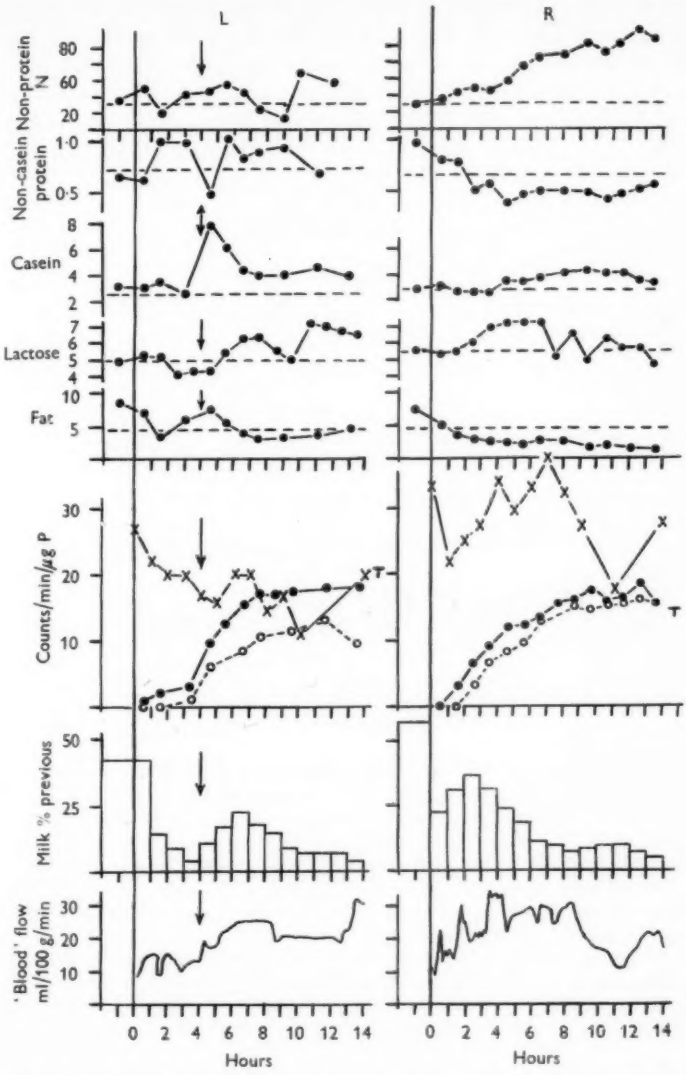

Fig. 6. Observations made in an experiment where one gland (L) was initially starved of acetate and glucose (10 % of normal level) and given normal amounts of amino acids, compared with the other gland given normal substrates throughout (R). At the arrow normal substrates were given. Glands weighing 550 g each and yielding 36·5 (L) and 34·5 (R) ml./hr before the experiment. Final weights 500 g (L) and 700 g (R). Glands removed under general anaesthesia (anoxic period 1 and 8 min) and perfused from 0 hr. Non-protein N, mg/100 ml.; remainder g/100 ml. ^{32}P specific activities: Inorganic P of 'plasma' (×), milk (●) and final tissue (T). Casein P (○).

During perfusion. Milk secretion can be stimulated by adding a substrate mixture 2–4 hr after the beginning of perfusion. Figure 4*a* illustrates an experiment in which one gland was given substrate from the beginning, whereas its fellow received none until after 4 hr perfusion. The milk yield of the starved gland fell to a minimum at 3–4 hr, and then increased in response to substrates and was maximal at 7 hr, after which

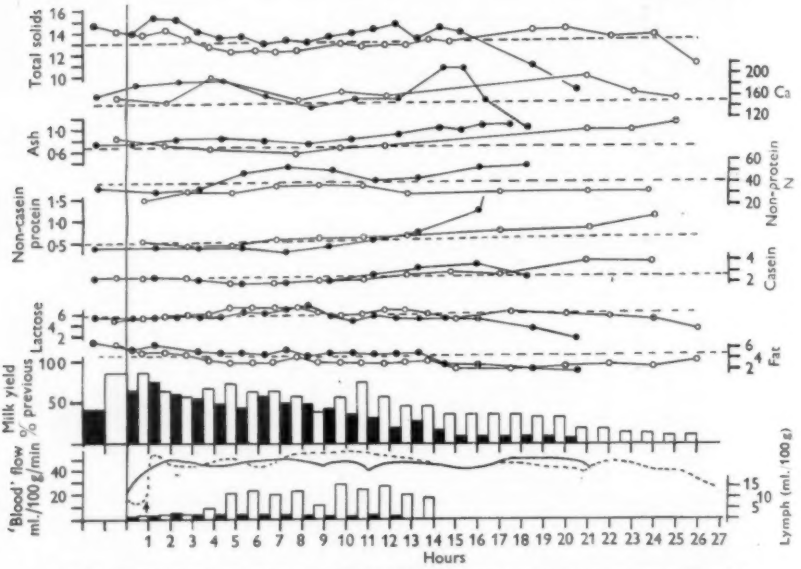

Fig. 7. Results of perfusing two glands (black L and white R) from one goat with dialysed defibrinogenated plasma-Krebs's solution using an artificial kidney. The 'blood' flow for the R side, interrupted lines; adenosine 100 mg was injected intra-arterially at the arrow. After 14 hr lymph flow was not measured but allowed to return to the perfusate. Oedema was observed at 7 hr.

Glands weighing 540 g (L) and 240 g (R) yielding 38 (L) and 9 (R) ml./hr before the experiment. Final weights 1152 (L) and 650 (R) g. Epidural anaesthesia (anoxic period 15 (L) and 10 (R) min).

Theoretically sufficient substrates run in to make 260 ml. (L) and 120 ml. (R) of milk; after the second hr 243 (L) and 92 ml. (R) were obtained.

Mean composition of milk 3 days before experiment indicated by horizontal broken lines. Ca and non-protein N, mg/100 ml; remainder g/100 ml.

the yield was identical with that of the fully fed gland. The total yield on the initially starved side was lower than that on the fed side. A similar experiment (Fig. 4*b*), in which substrates were added after an initial perfusion of 2½ hr, also showed the stimulation effect but in this case the total yield was not markedly reduced, compared with the other gland from the

TABLE 1. Milk yield and composition from glands perfused with substrates added. Average of seven glands giving 28 ± 16·5 ml./hr before the experiment. 'Previous' samples were bulked milk of the 3 days before perfusion. Sample 0 hr was the milk secreted during the operation for removal under epidural anaesthesia

			Hr perfusion										
		Previous	0	1	2	3	4	5	6	7	8	9	10
Yield	Av.	—	109·0	42·5	40·0	40·0	27·5	19·0	14·7	12·8	9·2	<8·0	<5·0
(% of previous)	S.D.	—	38·6	19·7	21·8	17·4	16·0	10·6	7·7	7·3	2·4	—	—
Lactose	Av.	5·08	4·36	4·90	5·15	5·45	5·45	5·49	5·55	5·46	4·61	3·43	2·53
(g/100 ml.)	S.D.	0·463	0·320	0·907	0·740	0·779	0·551	0·682	0·841	0·994	1·581	1·704	2·083
Fat (g/100 ml.)	Av.	4·18	8·47	5·86	5·23	3·79	3·65	3·84	3·87	4·00	3·99	3·91	3·33
	S.D.	0·725	2·855	3·155	2·806	1·951	2·167	2·441	2·613	2·200	1·820	1·690	2·349
Casein	Av.	3·14	3·17	3·30	3·19	2·84	3·05	3·20	3·42	3·75	3·71	3·60	3·67
(g/100 ml.)	S.D.	0·607	0·900	0·511	0·633	0·761	0·877	0·887	0·978	0·895	1·013	1·084	1·023
Total protein	Av.	3·56	3·72	3·72	3·56	3·21	3·50	3·64	3·76	4·32	4·53	4·70	4·42
(g/100 ml.)	S.D.	0·582	0·305	0·407	0·593	0·705	0·934	0·911	1·023	0·887	0·887	1·055	1·107
Ash (mg/100 ml.)	Av.	631	682	710	736	717	854	891	888	983	949	761	661
	S.D.	154	207	144	211	261	252	177	187	177	195	188	291

same goat. Comparisons such as these of the two glands from one goat give more precise information, because the within-goat variation of 'blood' flow and milk yield corrected for 'blood' flow is less than half the between-goat variation.

Perfusion of glands removed post mortem

In five experiments the animal was killed before removing the glands. This was done when we thought that removal under general anaesthesia might be detrimental to subsequent perfusion (see Methods). The results are quoted since the conditions are more comparable to those under which cows' udders from the slaughterhouse are perfused, in that the period between killing the animal and starting perfusion was 60–120 min (mean 90). On the other hand, cows killed in slaughterhouses are usually deprived of food and often of water and may be acutely distressed, whereas our goats were killed within a few minutes of removal from their normal environment. Three animals were killed in 12, 13 and 90 sec by rapid intravenous injections of pentobarbitone, one by a large CO_2 embolus (about 60 sec) and one by shooting through the head and subsequently bleeding (3 min). In two out of three pentobarbitone-killed animals the milk yields of the four glands were only 0·2, 0·1, 0·6 and 0·7 'hoursworth' in all, and this was all obtained in the first 3 hr; in the third animal more milk was obtained in the first hour but none thereafter. In the animal killed by embolism 0·9 and 1·2 'hoursworth' of milk was obtained. The glands of the shot animal gave 0·7 and 1·7 'hoursworth', but assessment was difficult because the milk was bloody from the start. However, none of these yields is significantly different from those of starved glands removed under epidural anaesthesia.

Secretion of radioactive phosphorus

Additional evidence about the formation of milk during perfusion was obtained by experiments in which labelled inorganic phosphate was added to the perfusate. There is much evidence that plasma inorganic phosphate is the main source of milk inorganic and casein phosphate in the whole animal (Folley, 1956). If this is true during a perfusion when radioactive inorganic phosphate is present in the perfusate, then at equilibrium the phosphate compounds in milk and tissue should have the same specific activity as the 'plasma'.

In five cases the isotope was added as $^{32}PO_4$ in Krebs's solution at the start of perfusion, producing a high initial specific activity of 'plasma' inorganic phosphate. The equilibration with the tissue was slower than expected. The 'plasma' specific activity at first fell rapidly (half-life 30–45 min) and thereafter more slowly (half-life 6–12 hr), partly owing to

the non-labelled P in the substrates being run in. In three other experiments we tried to keep the 'plasma' level constant by infusion of inorganic ^{32}P. The results were similar in all experiments. In the first 2 hr, whatever the 'plasma' level, the milk specific activity was always very low, and that of casein negligible, in spite of the addition of adequate substrates. This confirms that initially milk is formed from metabolites already in the tissue at the time of removal from the animal.

After 2 hr increasing concentrations of radioactive P appeared both in the milk inorganic P and, 1–2 hr later, in the casein P (Figs. 5*a* and 6). This occurred over a period of 5–8 hr. In three experiments the specific activity of the milk inorganic P and the casein P rose to equal the level of the 'plasma' inorganic P, thus showing that these milk constituents must have been formed from 'plasma' precursors. In the remainder, the milk inorganic P rose to only 50–90 % of the 'plasma' level and the casein P to somewhat less. However, the tissue specific activity of inorganic P at the end of these perfusions was always nearly equal to that of the milk inorganic P, but was somewhat lower than that of the 'plasma'. In an experiment where the milk yield of one gland before perfusion was very low (5 ml./hr compared with 19 ml./hr from the other), even after 9 hr the milk and tissue inorganic phosphate specific activity was only 22 % of the 'plasma' level, which had been kept reasonably constant (Fig. 5*b*). In this experiment lymph was collected in three 3-hr samples and in each case the specific activity was midway between that of milk and 'plasma', suggesting that there was a release of un-labelled phosphate in the tissue.

In another experiment, where the 'plasma' specific activity was kept constant, the glucose and acetate in the substrates given to one of the glands were reduced to 10 % of the normal level. The milk yield from this gland was only 7 % of normal in the third and fourth hours and the incorporation of ^{32}P into the milk was also low. After the fourth hour acetate and glucose were added at a normal rate. The milk yield increased immediately and was greater than the control at 6–8 hr. The incorporation of ^{32}P into the milk also increased sharply and actually reached equilibrium with the plasma before the control gland (Fig. 6).

Substrates and hormones necessary for milk secretion in vitro. At the start of this work we used a solution containing glucose, acetate, β-hydroxybutyrate, 20 amino acids, Ca and P (see Methods) together with insulin, thyroxine, and a bovine or goat anterior-pituitary extract or growth hormone, as the literature suggested that all these might be needed. Subsequently we omitted the hormones without observing any apparent reduction in milk yield. Although the experiments were not planned for statistical analysis, this was found possible by dividing the majority into the following groups: (*a*) substrates alone (i.e. glucose, acetate, β-hydroxy-

butyrate and amino acids), (*b*) substrates and thyroxine, (*c*) substrates, thyroxine, growth hormone and insulin, (*d*) substrates, thyroxine, insulin and miscellaneous substances. Multiple variant analysis by J. G. Rowell showed that, when blood flow and substrates given were allowed for, there was no significant difference between these groups, though there was between them and a further group when no substrates were given ($P < 0{\cdot}01$). Thus no clear benefit has been found from the addition to the perfusate (per hour) of glycerol 40 mg, propionate 40 mg, fumarate 40 mg, goat plasma 2 ml., emulsified fat 0·5 g, Mg 0·3 mg, insulin 0·5–1 u., growth hormone 1 mg, thyroxine 0·1–0·5 mg, cortisol 3–10 μg, aldosterone 10 μg, prolactin 0·5 mg, ascorbic acid 200 μg, or a crude anterior pituitary extract (equivalent to 0·3 g), in various combinations. Furthermore, no more milk was secreted in experiments, where we perfused with plasma + BOL (see Methods) or with dialysed plasma instead of albumin-Krebs's medium or in experiments where we added 25–50 ml. of the tissue-culture fluids Nos. 199 and 858 of Parker (see Biggers, Rinaldini & Webb, 1957). Recently we have also omitted β-hydroxybutyrate without detriment.

Gaseous exchanges

Metabolism of perfused cows' udders has been studied elsewhere by measuring the arteriovenous differences of O_2 and CO_2 and the R.Q. of glands receiving different substrates and hormones. However, the O_2 consumption has not always been estimated because the blood flow was not reported and the relationship of the gaseous exchange to the rate of milk secretion has not been assessed. This has now been done for the perfused goat udder.

In conscious lactating goats the O_2 uptake varies from $2{\cdot}34 \pm 0{\cdot}87$ ml./100 g/min to $1{\cdot}42 \pm 0{\cdot}4$ according to the milk yield and is $1{\cdot}09 \pm 0{\cdot}3$ in dry goats. The R.Q. is $1{\cdot}24 \pm 0{\cdot}23$ at all times during lactation and 1·01 in dry non-pregnant animals (Linzell, 1960*a*). The characteristically high R.Q. is a well known feature of functioning mammary tissue (Folley, 1949, 1956) and is generally accepted as indicative of fat synthesis.

In the present perfusions it was found that the arteriovenous differences tended to be inversely related to the rate of blood flow, whereas the O_2 uptake was often directly related to it. The latter is to be expected, because at very low flows large areas of the glands were not being perfused (see Methods). Nevertheless, the average figures are substantially in agreement with the other results as indicative of metabolic activity.

In the first 2 hr of perfusion, when the blood flow was generally increasing after the initial low level, the mean O_2 uptake was $0{\cdot}79 \pm 0{\cdot}42$ ml./100 g/min in glands receiving substrates, and 0·25 and 0·78 in two glands receiving none. The R.Q. was in the range of 1·07–1·87 in all cases.

In the period 2–6 hr glands still getting no substrates removed O_2 at a similar low rate, $0{\cdot}53 \pm 0{\cdot}35$ ml./100 g/min and although the average R.Q. was about 1, figures of 0 and ∞ were actually obtained. In the preliminary experiments on the basic perfusate, when the glands were given small amounts of glucose and acetate only, although the average R.Q. was 1·32, the O_2 uptake was only 0·5 ml./100 g/min. By contrast, glands receiving the full substrate mixture had an average R.Q. of $1{\cdot}5 \pm 0{\cdot}75$ and O_2 consumption of $1{\cdot}17 \pm 0{\cdot}57$ ml./100 g/min. After the 6th hour of perfusion, when milk secretion was declining in glands getting the full substrate mixture, the O_2 consumption fell to $0{\cdot}79 \pm 0{\cdot}64$ and the R.Q. to $1{\cdot}26 \pm 0{\cdot}28$.

Results with a modified technique

When sufficient substrates are given to higher-yielding glands the possibility arises of reaching unphysiologically high concentrations of the substances added. We have evidence from three experiments suggesting that this may actually inhibit milk secretion. We have therefore introduced an artificial kidney into the circuit. This consisted of 10 m of 4 mm diameter dialysis tubing (Visking) in 20 l. of Krebs's solution, containing amino acids, glucose and acetate at normal plasma levels and inserted between reservoir *B* and pump *B* (see Methods). In addition, we have used dialysed defibrinogenated plasma (Kekwick & Mackay, 1954) instead of albumin because frothing was less with the globulins present.

With these modifications we have obtained normal 'blood' flows throughout perfusion in six glands and the milk yields after the second hour have been 2·85–10·2 'hoursworth' (average 6·0) in experiments lasting 11–27 hr (average 16·6). The best result is shown in Fig. 7. We are now using this modified preparation in biochemical studies.

DISCUSSION

It is clear that in the study of milk secretion some problems can be most surely decided by perfusion experiments. This is reflected in the use of cows' udders from slaughterhouses by a number of laboratories, but only Tindal (1957) has compared the yield of milk obtained during the perfusion with that of the glands before the experiment. Since we have the facilities for doing this and for comparing the metabolic activities of perfused goats' udders with the glands of conscious animals from the same herd, our results should be of general interest.

The two most important questions to be answered are:

(1) How much of the milk obtained during perfusion is synthesized from precursors supplied?

(2) Is this sufficiently near the normal in quantity and quality to provide valid biochemical information?

In answer to the first question we find that little or none of the milk obtained in the first 2 hr is formed from added precursors but that later an increasing proportion is. In answer to the second question our results show that after the first 2 hr an average rate of milk secretion of 50 % for about 10 hr may be expected, provided that a normal blood flow is maintained and adequate amounts of substrates are given. The dependence of the milk yield on these two factors, which control the substrates available to the secretory cells, together with the fact that an increasing proportion of radioactive atoms are incorporated from the perfusate into the milk during this period, show that the preparation is behaving in a reasonable manner and is as suitable for biochemical studies as the tissue slice.

Associated with the reduced rate of milk secretion during perfusion are lower rates of lymph flow and O_2 consumption. Furthermore, the relationship in the majority of experiments between the amount of milk formed and the total substrates added shows that the perfused glands were only 15 % efficient and about 20 % of the glucose taken up could be accounted for as lactose. However, in the recent experiments with an artificial kidney, where fully normal blood flows were obtained, the over-all efficiency was about 50 % and about 35 % of glucose could be accounted for as lactose. This may be compared with an over-all calorie efficiency of 80 % in the conscious goat and transference of 50 % of glucose to lactose (Linzell, 1960*a*). It is interesting that guinea-pig mammary-gland slices transform 13 % of the glucose to lactose in 1·5 hr (Venkataraman & Reithel, 1957).

Although functional normality can only be finally decided by a detailed investigation of specific pathways, the simplest hypothesis at this stage is that the whole metabolism of the perfused gland is reduced. This may be due to each secretory cell working at a lower rate, or to fewer cells, each working normally. A reduction of blood flow generally means that areas of the gland are not being perfused adequately, so that fewer cells are functioning. When the blood flow is normal the milk secretion is generally still reduced, and since there are probably few arteriovenous anastomoses in the secretory tissue (Linzell, 1960*a*) this would suggest that all the cells are adequately supplied with substrates but are metabolizing at a reduced rate.

Although the oxygen consumption is generally reduced, when glucose and acetate are available the R.Q. is over 1, as it is in the conscious animal, and has been reported to be under similar conditions in the perfused cow's udder (Peeters & Massart, 1952). It should be pointed out, however, that the calculation of the R.Q. from instantaneous arteriovenous samples can

give misleading figures (0·4–3·76 in fed glands and 0–∞ in starved ones) and, clearly, integrated measurements of the O_2 consumption and the R.Q. are desirable.

It is not easy to determine precisely in each experiment how much of the milk obtained in the first 2 hr is preformed and how much is synthesized from tissue stores during perfusion. However, in four short preliminary experiments we found that after milking thoroughly with oxytocin 1·5–6·5 % of the weight of the tissue is milk on the basis of lactose estimation (it is immaterial whether this is in the cells or the alveolar lumina). Thus in spite of the thorough emptying of the glands with oxytocin at the start of perfusion, we can calculate from the weight of the tissue and its previous yield that most of the milk obtained in the first 2 hr could have been preformed. The appearance of radioactive P in the milk and casein does not help to decide how much is being synthesized in the first 2 hr, because of slow equilibration between perfusate and tissue, possibly associated with low initial blood flow. Nevertheless, the very small quantities of the isotope recovered in the milk in this period must be stressed. This, together with the fact that milk is obtained whatever the blood flow and whether or not substrates are present, shows that this initial period is not the most useful biochemically.

Therefore our results suggest that perfusion of cows' udders should be prolonged further than the usual 1–2 hr and that the milk obtained after this period should be analysed for isotopes. It is desirable in assessing the results to know not only the previous milk yield and the mean blood flow during perfusion but also the requirement of the gland for substrates, which may be calculated from the milk yield. The compositions of goat and cow milk, which are similar, indicate that about 4 g amino acids, 5 g glucose and 5 g acetic acid are required to form 100 ml. of milk exclusive of energy requirements. Measurements made in conscious goats (Linzell 1960*a*) show that in fact rather more glucose (10·3 g) and less acetate (3·2 g) are taken from the blood by the glands. Applying these figures to the work on lactose synthesis of Wood, Schambye & Peeters (1957), who added no amino acids, we find that the acetate and glucose added would make less than 100 ml. of milk. And yet in their perfusions, lasting 2–2½ hr, 1,140–1420 ml. of milk were obtained. Clearly these glands were synthesizing from tissue stores. The weight of tissue and the milk yield of cows' udders is so great that in many cases the problems of perfusing and of supplying sufficient substrates would bring the technique almost into the realm of chemical engineering.

An additional problem in the use of cows' udders is that they are usually obtained post mortem. The few experiments we have done in this way on the goat have not yielded significant amounts of milk after 2 hr. One

obvious factor is the long period of anoxia during preparation of the glands (1–2 hr in our experiments), but there may be others. Cooled glands removed from anaesthetized goats have withstood up to 27 min anoxia without detriment, so that the use of glands removed post mortem should be reinvestigated in both cows and goats.

The minimum substrate mixture necessary in our experiments has consisted of 20 amino acids, glucose, acetate, Ca and PO_4. The fact that we have been unable to demonstrate a beneficial effect of adding other components may mean that normal mammary tissue has a large store of hormones and of some substrates, that substances not investigated control the termination of the experiment, or that some of the substances tried blocked the beneficial effects of others. The gland, indeed, has considerable stores. For example, there is good evidence that neutral fat, in addition to acetate, is removed by cows' and goats' udders for fat synthesis. But the total milk fat concentration remained normal during most perfusions when no neutral fat was added and when it was, in two experiments, there was no benefit. In the whole animal labelled tristearin does not appear in the milk for 4–12 hr (Glascock, 1958), suggesting the existence of a large mammary store. However, in some experiments the fat content did fall markedly (e.g. Fig. 6) and in others the amount detectable in the cells at the end of perfusion was sometimes very low. Perhaps in such cases neutral fat would have been beneficial.

There may also be large stores of hormones in the tissue and possibly in the perfusate. Growth hormone and thyroxine stimulate secretion in the whole animal but have had no consistent effect in our perfusions, although, in two experiments, thyroxine and, in one, growth hormone have apparently stimulated one gland compared with the opposite gland. It is perhaps to be expected that the tissue has stores of hormones, because the half-life in the animal is long in relation to our perfusion. In the cow the half-life of thyroxine is 2·5 days (Pipes, Premachandra & Turner, 1959) and we have evidence from a decerebrate goat that it may be some hours for growth hormone. In the goat, section of the pituitary stalk cuts off much of the blood supply to the pituitary and the resulting necrosis causes a functional hypophysectomy (Daniel & Prichard, 1958). We have found that necrosis occurs after stalk section during decerebration, and in a lactating decerebrated goat followed for 25 hr milk secretion did not fall below the previous yield until after 8 hr.

To make more complete studies of milk secretion in isolated glands, particularly in relation to substances of which they have large stores, it is thus clearly desirable to increase the functional life to at least 24 hr in every experiment. This may be possible because the glands continue to remove O_2 and substrates after milk production has ceased. At present

the limiting factors are certainly multiple. However, the accumulation of toxic factors such as ammonia and urea would not seem to be involved because changing the perfusate completely at 6 hr in one experiment when the yield was still 20 % did not produce any noticeable improvement. Nevertheless, irreversible changes may have already occurred. The incorporation of an artificial kidney into the apparatus reduces the chances of this because it increases the effective volume of the perfusate for small molecules 20 times. It is important, however, to note that glucose and acetate diffuse through such membranes more slowly than Na^+ and therefore tend to accumulate in the perfusate. Further, diffusible substances from the tissue may be reduced in concentration to below a critical level. The recent good experiments indicate that this is not at present a problem, although in preliminary experiments with the kidney a marked terminal fall in milk lactose was associated with a low terminal tissue uridine monophosphate concentration.

Since we have no evidence of toxic accumulations it may be that secretion stops because some unknown factors are lacking. Glands perfused with plasma, dialysed plasma or 5–10 % of plasma or tissue culture fluid in the perfusate did not secrete more milk. Again, this does not rule out the lack of a metabolite with a high rate of utilization which we have not added or added at too low rate.

Finally, our results show that the perfused mammary gland is capable of yielding useful information about metabolism if the results are interpreted with caution. For a proper assessment of any experiment it is necessary to know at least the previous milk yield, the mean blood flow during perfusion and the exact quantities of substrates needed, given and used.

SUMMARY

1. Goat udders removed under anaesthesia have been perfused with washed homologous goat red cells in Krebs's medium plus albumin or albumin and globulin for up to 27 hr.

2. In glands perfused entirely without substrates the mean blood flows throughout perfusion were in the normal range for conscious goats: 40 % of the hourly milk yield before the experiment was obtained in the first 2 hr but very little after that. After 2 hr lactose and fat concentrations fell but that of casein was maintained or tended to rise.

3. When a substrate solution of glucose, acetate, amino acids, Ca and P was run in during perfusion, significantly more milk was obtained after 2 hr and secretion remained above 5 % for a further 2–25 hr. Milk composition generally remained essentially normal.

4. After the second hour the milk yield was related to the amount of substrates added and, even after 4 hr starvation, glands responded to their

addition. The milk yield was also related to the mean blood flow and the difficulties of keeping this in the normal range are discussed. The average milk yield for all levels of blood flow and substrates added was 17 % of the previous level, but when normal flows are maintained and adequate substrates given an average of 50 % may be expected for some hours.

5. Inorganic $^{32}PO_4$ added to the perfusate did not appear in milk inorganic PO_4 in significant quantities in the first 2 hr and was not maximal until 5–8 hr. ^{32}P appeared in casein 1–2 hr later but reached 50–100 % of the milk and tissue inorganic PO_4 specific activity.

6. No consistent benefit was observed from adding many other substrates or hormones, but other conditions affecting the perfusion are discussed.

7. We conclude that the glands when removed have a large store of substrates and possibly hormones and that only the milk produced after 2 hr is formed from added precursors. The uptake of ^{32}P confirms that milk obtained later is so formed. Mean blood flow and the quantity of substrates needed and given must be allowed for in assessing milk yield during perfusion.

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RECEPTIVE FIELDS OF OPTIC NERVE FIBRES IN THE SPIDER MONKEY

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Our present knowledge of how mammalian retinal ganglion-cell receptive fields are organized is based mainly on findings in the cat by Kuffler (1953). These results have since been confirmed and extended (Barlow, FitzHugh & Kuffler, 1957; Hubel, 1960; Wiesel, 1960), but up to now similar studies have not been made in primates. The retina of the monkey is of interest, since in most species, including *Ateles* (spider monkey) and *Macaca mulatta*, it is deeply pigmented and has a well defined fovea. It appears to be much closer to the human retina than to that of the cat, which has a highly reflectile tapetum and lacks a fovea. The purpose of this report is to describe the receptive fields of single optic nerve fibres in the spider monkey. In view of the monkey's ability to discriminate colours, some observations were also made on ganglion cell responses to monochromatic stimuli.

METHODS

The four young spider monkeys (8–10 lb., 3·6–4·5 kg) in this study were prepared in a manner similar to that described for the cat (Hubel & Wiesel, 1959). An animal was anaesthetized with thiopental sodium and light anaesthesia was maintained throughout the experiment. The head was placed in a Horsley–Clarke stereotaxic instrument designed to permit stimulation of almost any part of the visual field (Talbot & Marshall, 1941). The eyes were immobilized with the muscle relaxant succinylcholine: this made it necessary to use artificial respiration. The eyelids were held open and contact lenses protected the corneas. The pupils were dilated and accommodation was relaxed with atropine. A slit retinoscope was used to determine the correct supplementary lenses for focusing the eyes on a screen at a given distance.

The animal faced a large tangent screen. This was generally placed 1·5 m from the monkey, but for the smallest stimuli it was moved to a distance of 10 m. A distant tungsten lamp supplied a diffuse background light which produced on the screen a variable luminance of up to 2·0 $\log_{10}$ cd/m^2. Circular or annular spots provided by a tungsten projection lamp could be shone on different parts of the screen. The maximum spot luminance was 3·0 $\log_{10}$ cd/m^2. Stimulus spots were adjusted in intensity with an iris diaphragm so as not to exceed the background by more than 2 $\log_{10}$ units. At 10 m distance spots were obtainable subtending angles down to about 20 sec of arc, equivalent to about 2 μ on the retina. Fifteen interference filters (Farrand Optical Co., New York, N.Y.) ranging from 400 to 700 mμ with a band width at half maximum transmission of 25–50 mμ, were used to obtain coloured

stimuli. The coloured lights were not calibrated for equal energy, but this was not necessary for the observations to be described.

By a projection method points corresponding to the optic disk and the fovea were mapped out on the screen. An ophthalmoscope was designed which permitted direct vision of the fundus by means of a small spot of light. This spot was directed through the centre of the pupil at the fovea or optic disk and the instrument was locked in position. The ophthalmoscope shone a second beam in a direction exactly opposite to the retinal beam, making a small spot on the screen. The position of this spot then corresponded to the illuminated retinal point. Points on the screen could thus be specified with reference to the fovea and the optic disk, and points on the retina were stimulated by illuminating the corresponding points on the screen. The correspondence could be verified by illuminating a point on the screen (e.g. that corresponding to the optic disk) and observing the retina directly with an ordinary ophthalmoscope through a half-silvered mirror placed at 45° in front of the eye.

Regions in the visual field which gave 'on' or 'off' (excitatory or inhibitory) responses to illumination were marked on sheets of paper fixed to the screen. These provided permanent records of the size and shape of the receptive fields and of their position with respect to the fovea and the optic disk.

Single fibres were recorded from the optic nerve with tungsten micro-electrodes (Hubel, 1957). Cathode-follower input and a condenser-coupled pre-amplifier were used in a conventional recording system. In preliminary experiments, not included in this series, the nerve was approached under direct vision from below by a transpharyngeal route and from above by removing one frontal lobe. Both procedures proved tedious, and neither gave good stability. The method finally adopted was a modification of a technique used for single-unit recording within the brain of the unrestrained animal (Hubel, 1960). The nerve was approached from above through the intact brain. A closed system was used to lessen vascular and respiratory pulsations. A hydraulic micro-electrode positioner was oriented by Horsley–Clarke stereotaxic methods. The co-ordinates of the optic nerve were determined from the position of the bony optic foramen, using the average from several spider monkey skulls. During the actual experiment a small hole was made in the skull and an 18-gauge steel tube attached to the positioner was lowered through the brain until its tip was several millimetres above the optic nerve. The space between the tube and the skull was sealed with dental-impression cement. The electrode, held within this outer tube by an insulated 26-gauge hollow needle, was then hydraulically advanced until it entered the optic nerve.

RESULTS

One hundred and twelve optic nerve fibres were studied. In the light-adapted state all fibres showed maintained activity; that is, they discharged impulses in the absence of any stimulus besides the steady uniform background light. No systematic observations were made in the dark-adapted state. Firing patterns resembled, at least superficially, those described for the cat's ganglion cell by Kuffler, FitzHugh & Barlow (1957).

If a small spot of light was projected on the screen one could always find a restricted area over which firing of a fibre could be influenced. This was termed the receptive field of the fibre. Receptive fields had the same general characteristics as those of the cat, described by Kuffler (1953). As in the cat, two main types could be distinguished, both concentrically arranged, one with an 'on' centre and an 'off' periphery, the other with an 'off' centre and an 'on' periphery. Records from an

'off' centre unit are shown in Fig. 1, and illustrate suppression of firing with an 'off' discharge in response to a centred spot, and an 'on' discharge in response to an annulus. Responses from the periphery of receptive fields were often difficult or impossible to elicit in the monkey when stimulus and background intensities were of the same order as those used in work on the cat's retina. However, if both the stimulus and the background luminances were increased by about 2 log units, peripheral responses could usually be obtained. The influence of peripheral parts of receptive

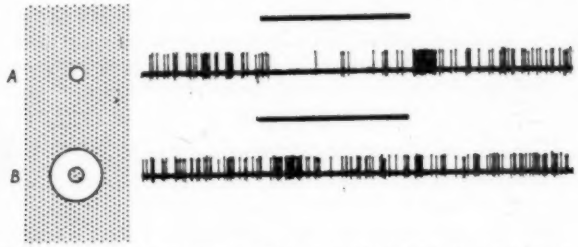

Fig. 1. Responses of an 'off' centre unit to restricted light stimulation. In each example the upper line indicates when the stimulus light is on. *A*, ½° spot of light shone in the centre of the receptive field; *B*, annulus 5° in outside diameter, 2° inside diameter, with its centre over that of the receptive field. Receptive field located on the retina 28° above and 12° nasal to the fovea. Duration of stimuli, 1·5 sec.

fields could be demonstrated even with weaker stimuli, since it was always possible to decrease a centre response by simultaneous stimulation of regions outside the field centre. In Fig. 2 spots of successively larger size were used to stimulate an 'on' centre fibre. The smallest spot did not fill the centre (Fig. 2*A*), and it gave a response considerably weaker than that evoked by a second spot (*B*) which just filled the centre. A still larger spot invaded the periphery (*C*), and the response was now less marked than with the second.

The receptive fields studied were situated in various parts of the retina, ranging between 4° and 56° from the fovea. In any given penetration through the optic nerve from above there was a tendency for receptive fields of the first fibres recorded to be located in the lower quadrants of the visual field. As the electrode was lowered the receptive fields tended to be located higher and higher in the visual field. Otherwise, however, there was little order in the position of fields, and fields of successively recorded fibres were often separated by a considerable distance, in an unpredictable fashion. This is illustrated in Fig. 3 for a particular penetration in which 20 fibres were mapped. From this and other penetrations one had the impression that there was no very detailed topographical

representation in this part of the optic nerve, but only a coarse segregation of fibres from the different quadrants.

The size of receptive field centres ranged from 4 min to 2° of arc. The centres were mapped out by using spots of light which for each field were chosen so as to be small relative to the size of the centre. Relatively high background illumination (1·0 $\log_{10}$ cd/m^2 or more) was used to minimize effects of scattered light. No attempt was made to determine accurately the total extent of each receptive field, but there was little doubt that for

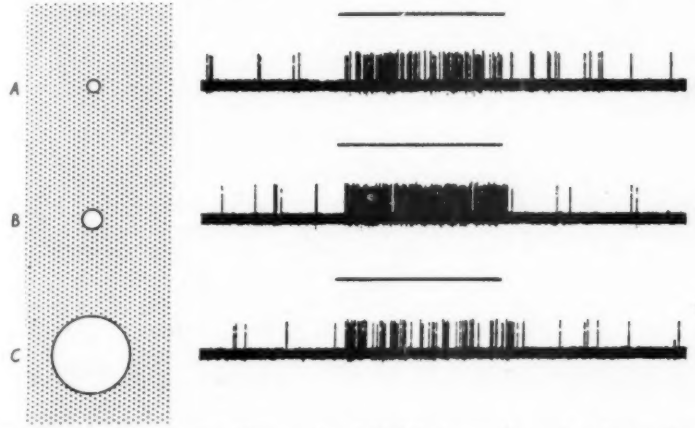

Fig. 2. 'On' centre unit with receptive field 11° above, 4° nasal to fovea. Responses to centred spots $\frac{1}{8}$°, $\frac{1}{2}$° and 4° in diameter. Duration of stimuli, 1·5 sec.

most fields the total diameter was many times that of the centre. There was a clear tendency for receptive field centres near the fovea to be smaller than those in the periphery, although there was considerable variation in centre size at any given distance from the fovea. In Fig. 4 the diameters of 65 field centres are plotted against distance from the fovea. Both 'on' and 'off' centre units were well represented in the series. The number of units is too small to justify any conclusions about the preponderance of one type of field over the other. In particular, the apparent predominance of 'on' centre units over 'off' centre in the region near the fovea is of interest, but again, conclusions can hardly be drawn from such a small sample.

In the present work there was no attempt to make a thorough study of responses to coloured stimuli. However, some preliminary results showed that there are ganglion cells which respond in specific ways to colour. An example is given in Fig. 5. In this unit a 2° spot of white light gave a weak response consisting of suppression of firing followed by a feeble 'off'

discharge (Fig. 5*A*). A decrease in intensity of the white light produced even weaker 'off' responses. With the light source intensity set as in Fig. 5*A*, a blue interference filter was placed in front of the projector. Each time the blue spot of light was shone on the screen it produced a strong 'on' response (Fig. 5*B*). If a red filter was substituted for the blue the firing was strongly suppressed during the stimulus and this was

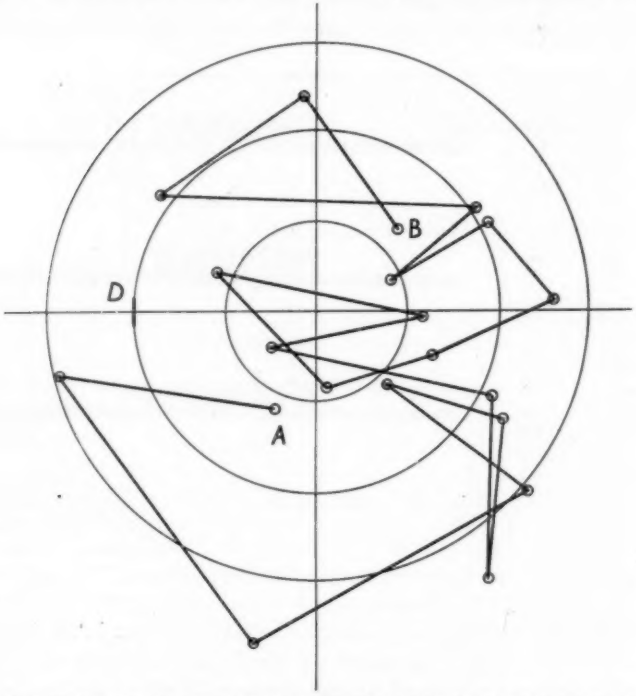

Fig. 3. Location on the visual field of receptive fields of 20 optic nerve fibres recorded in sequence as the electrode was advanced vertically from above through the left optic nerve. The vertical and horizontal lines represent the vertical and horizontal meridia; these cross at the fovea. The optic disk *D* is situated in the temporal field 20° from the fovea. The first unit, *A*, had its receptive field in the lower temporal quadrant of the visual field; the last, *B*, in the upper nasal quadrant.

followed by an 'off' discharge (Fig. 5*C*). It is clear that either filter greatly reduced the radiant flux of the light beam, yet the effects of the stimulus were much enhanced. Moreover, the responses to stimulation with the two different colours were of opposite type. This was true regardless of stimulus intensity or spot size. Two other units studied with monochromatic light gave 'on' responses to wave-lengths shorter than

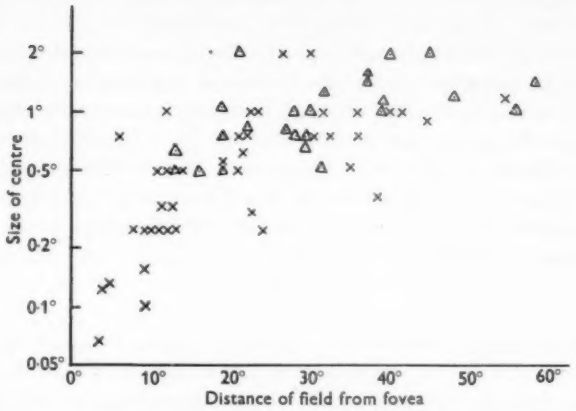

Fig. 4. Diameters of receptive field centres in degrees (logarithmic scale), plotted against distance in degrees of each field from the fovea. ×, 'on' centre units; △, 'off' centre units.

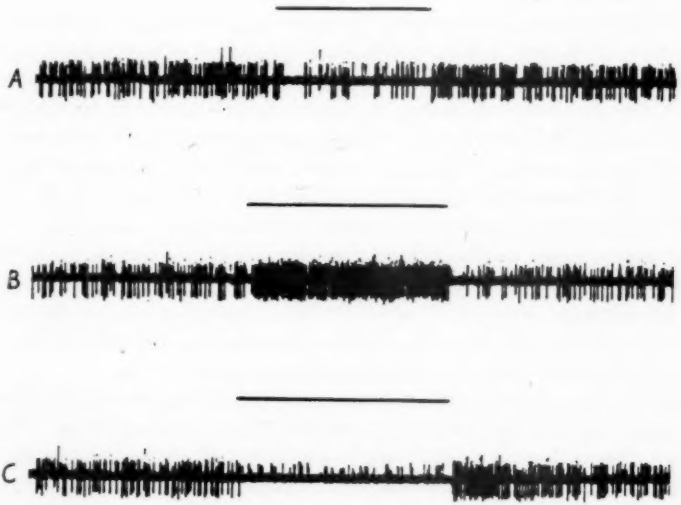

Fig. 5. Response of optic nerve fibre to 2° spot of light shone in centre of receptive field. Location of field, 7° below, 29° temporal to fovea. *A*, white light; *B*, same as *A*, but with blue interference filter (peak transmission 480 mμ) inserted between projector and screen; *C*, same as *A*, but with red interference filter (peak transmission 630 mμ) inserted between projector and screen. Duration of first stimulus, 1 sec.

498 mμ and inhibition with 'off' responses for longer wave-lengths. With 498 mμ a very feeble 'off' response was produced.

It is likely that the units with specific colour responses described above form a small minority of the population of optic nerve fibres, since a number of additional units studied with monochromatic stimuli gave centre responses of constant type, either 'on' or 'off', for all wave-lengths. In these white light gave brisk responses. In this type of ganglion cell, however, there were indications of some variability in the position of spectral sensitivity peaks from one unit to the next, suggesting that they too, conveyed information on colour.

DISCUSSION

From the results presented in this paper it is clear that in the monkey, as in the cat, the receptive fields of retinal ganglion cells are of two main types, one with an 'off' centre and an 'on' periphery, the other with an 'on' centre and an 'off' periphery. The antagonism between centre and periphery of a receptive field is generally not so complete that diffuse light is ineffective.

Fields in the vicinity of the monkey fovea tend to have smaller centres than those in the periphery (Fig. 4). A similar finding has been reported for the cat (Wiesel, 1960). Differences between central and peripheral visual acuity in man may well be related to variations in receptive-field centre size similar to those found in the monkey and cat. In the present study the smallest centre was found for a receptive field 4° from the fovea; this had a diameter of less than 4 minutes of arc. No recordings were made from fibres with receptive fields in the fovea, but it is likely that even smaller field centres are present in this region, since there is less convergence in the pathway from receptors to ganglion cells in the foveal region than in other parts of the retina (Polyak, 1957). Our failure to find foveal fields is probably related to the small diameter of the macular fibres. Moreover, the macular bundle occupies a small part of the optic nerve, and could easily be missed in random penetrations.

The cat has a highly reflectile tapetum behind the retina; in contrast, the retina of the spider monkey is deeply pigmented. With identical stimulus conditions it was generally more difficult to produce a response from the receptive-field periphery in the spider monkey than in the cat. However, it was found that by increasing the stimulus and background luminance by two logarithmic units peripheral responses could consistently be obtained. It is probable that the tapetum of the cat increases the effectiveness of the background illumination, and this may be necessary for the production of a good response from the periphery of the receptive field. It is known, for example, that the influence of the periphery on the

centre response becomes more pronounced with increasing background illumination (Barlow *et al.* 1957).

Several authors have described responses of opposite type to stimulation with light of different colours. Svaetichin (1956) demonstrated membrane hyperpolarization with short wave-lengths and depolarization with long wave-lengths in recordings from cells in the inner nuclear layer of the fish retina (MacNichol & Svaetichin, 1958). Similar results have been reported by other workers (Motokawa, Oikawa & Tasaki, 1957; Tomita, Tosaka, Watanabe & Sato, 1958). In the lateral geniculate body of the rhesus monkey de Valois, Smith, Kitai & Karoly (1958) described units responding with an 'on' discharge to blue light and an 'off' discharge to red light. Wagner, MacNichol & Wolbarsht (1960), recording spike discharges from ganglion cells in the goldfish retina, have since found similar discharge patterns to coloured stimuli.

In the present study of the spider monkey's extra-foveal retina three ganglion cells showed colour responses analogous to those described above. The presence of maintained discharges made it possible to demonstrate inhibitory effects during long wave-length stimuli, in addition to the excitatory responses to short wave-lengths. White light was less effective than monochromatic light, provided the wave-length was either longer or shorter than about 500 mμ. The decreased responsiveness to white light which has been noted also by de Valois (1960) is presumably due to the antagonistic effects of monochromatic light of short and long wave-lengths. This is strikingly similar to the way in which form specificity is obtained in the visual system; shining a restricted light on appropriate parts of a receptive field produces excitatory or inhibitory responses, and the effects tend to cancel when opposing regions are stimulated simultaneously, for instance with diffuse light. In both cases an unspecific stimulus produces less effect on the firing of a single cell than one restricted in form or wave-length, even though it contains more energy.

SUMMARY

1. Receptive fields of retinal ganglion cells were studied in the light-adapted spider monkey. All fields mapped with white light had a concentric arrangement similar to that of cat retinal ganglion cells, with a sharply demarcated 'on' centre surrounded by an antagonistic 'off' periphery, or the reverse.

2. The smallest receptive field centres were found near the fovea, and the size of centres tended to increase with increasing distance from the fovea. The smallest centre had a diameter of 4 minutes of arc (corresponding to about 20μ on the retina) and was located 4° from the fovea; the largest centre had a diameter of 2°.

3. Three ganglion cells out of about 100 responded in a specific way to coloured stimuli. In these cells light of short wave-length produced an 'on' response and light of long wave-length evoked inhibition followed by an 'off' response. Transition between the two types of response occurred at about 500 mμ; light of this wave-length evoked only feeble 'off' responses. Very weak responses were obtained to white light, presumably owing to the antagonism between light of short and long wave-lengths.

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SOME PROPERTIES OF AVIAN SKELETAL MUSCLE FIBRES WITH MULTIPLE NEUROMUSCULAR JUNCTIONS

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It has recently been found that certain skeletal muscles of the bird contain fibres which are innervated at many points (Ginsborg, 1960; Ginsborg & Mackay, 1961). The purpose of this paper is to describe some of the characteristics of these fibres. In particular, it will be shown that they are innervated by several axons, that they are able to produce propagated action potentials and that they respond to prolonged depolarization with a prolonged contracture. The experiments have been mainly carried out on the chick, either on single multiply-innervated fibres of the isolated biventer cervicis, or on the isolated anterior latissimus dorsi nerve-muscle preparation. The advantage of the latter preparation is that the anterior latissimus dorsi is composed largely, or perhaps entirely, of fibres with multiple neuromuscular junctions (Ginsborg & Mackay, 1961). The properties of such fibres may therefore be inferred from the properties of the muscle as a whole. Some of the results have been briefly reported (Ginsborg, 1959; Ginsborg & Mackay, 1960).

METHODS

The preparations were removed from chicks (Brown Leghorns) about 3 weeks old. In two experiments latissimi dorsi were taken from a pigeon and a rook, both apparently adult, but of unknown age. The birds were previously anaesthetized with I.V. Na phenobarbitone solution 9 g/100 ml., 2 ml./kg, or decapitated. The biventer cervicis preparation has already been described (Ginsborg, 1960; Ginsborg & Warriner, 1960). The latissimi dorsi of the chick (Fig. 1) and the rook are in two separate parts, an anterior and a posterior, lying immediately beneath the skin and extending between the mid line and the humerus. In the pigeon the posterior part is not present. The anterior latissimi dorsi were removed together with part of the humerus to which the peripheral end is attached. The nerve supply to the muscle is contained in three separate trunks which lie side by side for some way. For electrical recording, one branch (*a*), which appears to innervate part of the 'central' end of the muscle, was generally cut in the course of removing the connective tissue lying over the surface of the muscle, in order to avoid artifacts.

Intracellular recording. The biventer cervicis was mounted in Krebs–Henseleit solution (1932) as has been described previously (Ginsborg, 1960). The methods for recording from and polarizing single fibres with micro-electrodes were conventional (Fatt & Katz, 1951). The nerve was freed from the cranial tendon which encapsulates it (Ginsborg & Warriner,

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1960) and the cut end was sucked into a capillary electrode (Furshpan & Potter, 1959). Stimuli were applied via an RF isolating unit (Schmitt, 1948) to minimize stimulus artifacts.

To avoid breaking an excessive number of electrodes it was necessary to reduce the muscle twitch which occurred on nerve stimulation. The experiments were therefore made at room temperature, and parts of the muscle were often made unresponsive to nerve stimulation either by cutting intramuscular branches of the nerve or by replacing part (up to 80 %) of the Na content of the bathing solution with tris-(hydroxymethyl)-amino-methane chloride (Lüttgau & Niedergerke, 1958).

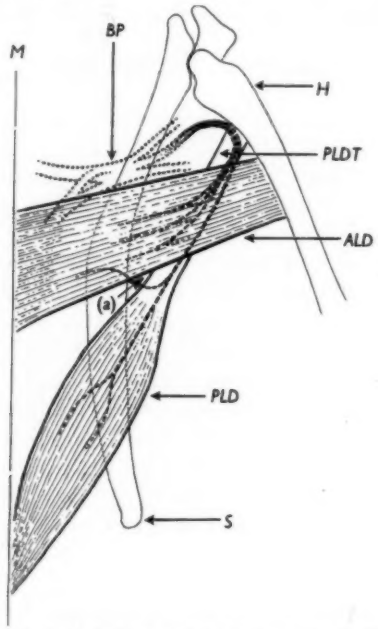

Fig. 1. Diagram of dorsal view of right latissimus dorsi of chick. The nerve supply is exposed by detaching the 'central' end of the muscles from the supraspinous ligament on the mid line and reflecting them about the humerus. Part of the scapula overlying the brachial plexus is then removed and the branches of the median nerve which supply the muscles may then be freed from the surrounding connective tissue. *ALD*, anterior latissimus dorsi; *BP*, brachial plexus; *H*, humerus, *M*, mid line; *PLD*, posterior latissimus dorsi; *PLDT*, tendon of *PLD*: *S*, scapula; *a*, see text.

Extracellular recordings were made from latissimus dorsi muscles with either platinum electrodes connected to the input of an a.c. amplifier, or with Ag–AgCl–agar wick electrodes connected to the input of a d.c. amplifier. For a.c. recording the muscles were usually mounted vertically on a multi-electrode assembly consisting of a rectangular Perspex frame into which ten horizontal platinum wires 4 mm apart were sealed. The nerve was threaded through a hole in the frame into which two concentric platinum annuli, which served as stimulating electrodes, were sealed. The preparations were mounted in a closed chamber containing Krebs–Henseleit solution, which was drained only for the short periods

required for recording. For d.c. recording one wick electrode was usually kept fixed at one end of the muscle, and the bathing fluid surface, below which dipped the second wick, served as the other recording electrode (Fatt, 1950). The preparations did not appear to deteriorate over periods of several hours.

Isometric tension changes were recorded conventionally with an R.C.A. 5734 mechano-electric transducer coupled either to an oscilloscope or to a pen recorder.

RESULTS

Intracellular recording from fibres with multiple end-plates

Multiple innervation. Figure 2 illustrates the response to nerve stimulation of three fibres of the biventer cervicis in which the miniature end-plate potentials were characteristic of muscle fibres with many neuro-

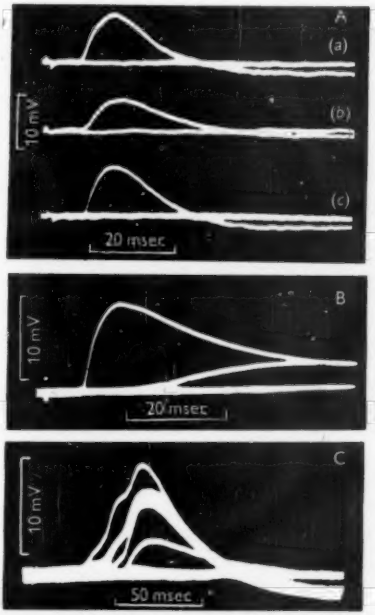

Fig. 2. Intracellular records of responses of three multiply-innervated fibres of biventer cervicis muscles *A*, *B* and *C*, to graded stimuli applied to the nerve. Temperature, 19° C. Movement of muscles reduced in *A* by cutting several intramuscular nerve branches, in *B* by replacing 80 % NaCl in bathing solution by tris-(hydroxymethyl)-amino-methane Cl. In *A* responses (*a*) and (*c*) were evoked by stimuli of equal strength.

muscular junctions along the fibre (see Ginsborg, 1960). It was evident that such fibres were innervated by several axons.

In several fibres the smaller responses, i.e. those to smaller stimuli, which therefore resulted from the stimulation of larger-diameter and more

rapidly conducting motor axons, occurred with a longer latency than those to the larger stimuli (Fig. 2*B* and *C*). This result suggests that very extensive branching of these axons occurs or that different axons supplying the same muscle fibre have very different lengths.

Action potentials in response to nerve stimulation. Of the seventy-one multiply-innervated fibres of the biventer cervicis muscles which were

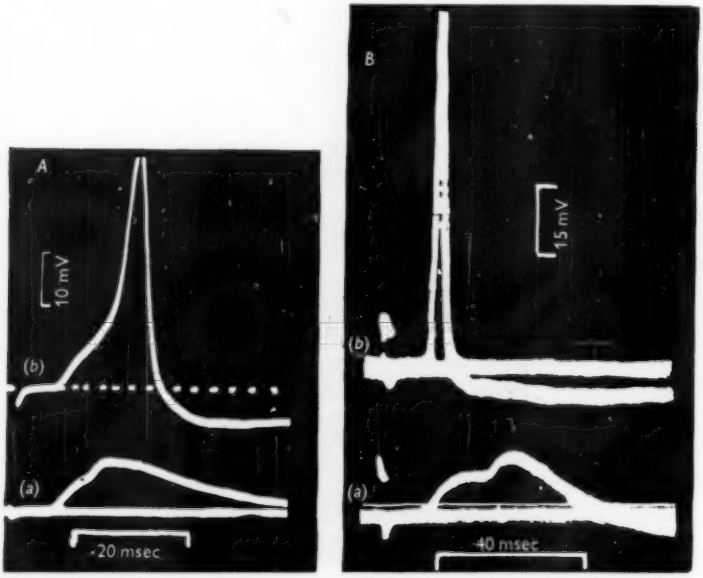

Fig. 3. Intracellular records of responses to submaximal (*a*) and maximal (*b*) nerve stimulation of two fibres, *A* and *B*, of biventer cervicis muscles. Temperature, 19° C. Muscle bathed in Krebs–Henseleit solution with 80 % NaCl replaced by tris-(hydroxymethyl)-amino-methane Cl.

tested only seven were observed to respond to maximal nerve stimulation with action potentials. Two examples are shown in Fig. 3. From the results to be described below it seems likely that the absence of action potentials in the remainder was due, not to a genuine inability to produce action potentials, but to the measures taken to reduce the muscle twitch (see Methods) and to the reduction in resting potential caused by the insertion of the micro-electrode.

Response to direct stimulation. Whenever it was possible to insert a second, polarizing, micro-electrode into a multiply-innervated fibre, it was possible also by some means to evoke an action potential. In three of nineteen fibres an electrotonic depolarization was effective (Fig. 4*A*).

Although measurements of resting potential were unreliable (see Methods in Ginsborg, 1960) in these three fibres the resting potentials appeared to be above 56 mV. The remaining fibres appeared to be in a state of 'cathodal depression' (see for example, Lorente de Nó, 1947), since they could only be stimulated either by 'anode-break' excitation (Fig. 4 B ii) or by applying an electrotonic depolarization after raising the resting potential at the site of the micro-electrodes by means of a steady hyperpolarizing current.

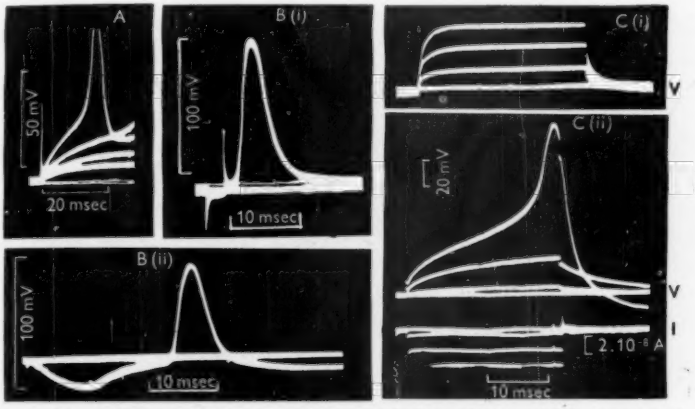

Fig. 4. Intracellular records of action potentials from three multiply-innervated fibres, *A*, *B*, *C*, of the biventer cervicis. Temperature, 18° C. *A*: resting potential, 66 mV; fibre stimulated with internal polarizing electrode close to recording electrode; three subthreshold responses also shown. *B*: (i) resting potential, 83 mV; stimulation by second micro-electrode external to fibre and 1 mm away from recording electrode; (ii) resting potential, 52 mV after insertion of polarizing electrode into the fibre; it could no longer be stimulated by depolarization, but responded to anode break excitation. Identical voltage and time scales in (i) and (ii). *C*: V, voltage; I, current; (i) resting potential, 58 mV; no action potential in response to depolarization; (ii) resting potential set to 100 mV by steady current (not shown) passed inwards across membrane; a sufficiently large outward current pulse (I) stimulated the fibre. Identical voltage and time scales in (i) and (ii).

Many of the responses, both end-plate and action potentials terminated with a phase of hyperpolarization (Figs. 2–4). It is very likely that this feature although involving 'delayed rectification' (Hodgkin, 1951; cf. Burke & Ginsborg, 1956), was associated, like the inability to produce action potentials, with the depression of the resting potential caused by inserting micro-electrodes into fibres of small diameter and therefore of high resistance (cf. Katz & Thesleff, 1957; Koketsu & Nishi, 1957*b*). This is strongly suggested by the experiment illustrated in Figs. 4 B (i) and (ii). In this fibre with a high resting potential, 83 mV, an action potential was evoked by external stimulation (i.e. by an external micro-electrode closely applied to the fibre and made negative with respect to the bath). This action potential was not followed by a phase of hyperpolarization (Fig. 4 B i). When the polarizing electrode was inserted into the fibre (Fig. 4 B ii) the resting potential fell by 31 mV and the fibre failed to respond to direct depolarization. It was, however, stimulated by the break of an anodic pulse, and the action potential was now followed by a phase of hyperpolarization.

Extracellular recording

The preceding results suggest that the multiply-innervated fibres in the muscles of the chick can produce propagated action potentials and that at least some of them do so on maximal nerve stimulation. More convincing evidence is provided by the results of experiments made on the anterior

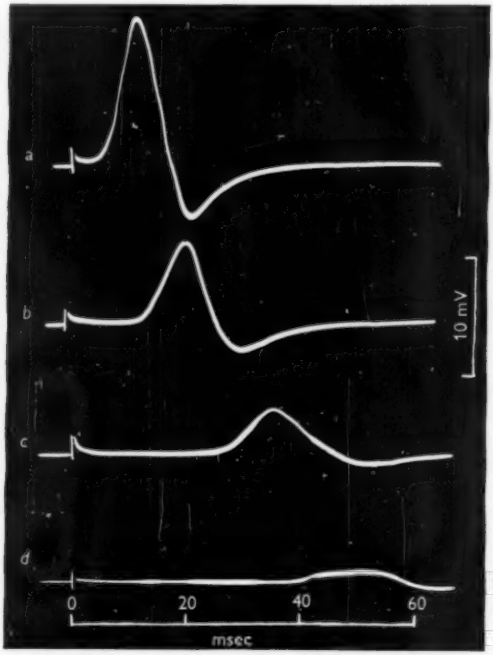

Fig. 5. Action potentials recorded externally from an anterior latissimus dorsi muscle in response to direct stimulation (neuromuscular transmission blocked by tubocurarine Cl 8×10^{-6} M). 21-day-old chick, length of muscle 23 mm. Temperature, 28° C. Stimulating electrodes 2 mm apart at peripheral end of muscle. Recording electrodes 3 mm apart; distance between inner recording and stimulating electrodes: *a*, 2 mm; *b*, 6 mm; *c*, 12 mm; *d*, 18 mm. Stimulus artifacts reinforced with short vertical lines.

latissimus dorsi, which has been shown to consist largely, or perhaps exclusively, of fibres with multiple neuromuscular junctions (Ginsborg & Mackay, 1961).

Response to direct stimulation. Figure 5 shows unequivocally that the fibres of the anterior latissimus dorsi are able to conduct action potentials and that at least some fibres extend the length of the muscle. The ampli-

tude, 14 mV, of the largest externally recorded action potential from the muscle was small, as compared, for example, to the amplitude of the action potential which can be recorded under favourable conditions from the frog's sartorius muscle (50–60 mV, Katz & Kuffler, 1941), but the fibres of the anterior latissimus dorsi of the 21-day-old chick are less than 20 μ in diameter, compared with 73 μ in the frog's sartorius (Katz, 1948, p. 523); and since the muscle was kept moist by vigorous bubbling with gas of the bathing fluid at the bottom of the chamber containing the muscle its extracellular resistance was probably low: a relatively small amplitude of the externally recorded action potential would therefore be expected (Hodgkin & Rushton, 1946).

The speed of conduction along the muscle was low; in six muscles, at temperatures between 28 and 34° C, the average speed of conduction varied between 0·41 and 0·70 m/sec. The slow speed of conduction cannot be entirely due to the small diameter of the fibres (Hodgkin, 1954) since the posterior latissimus dorsi, which is composed of fibres of somewhat smaller diameter, conducts at the rate of 2·3–2·8 m/sec (four muscles, 31–36° C).

Response to nerve stimulation. In Fig. 6 the responses to submaximal and maximal nerve stimulation of an anterior latissimus dorsi muscle are compared with the response of the same muscle to direct stimulation. The response to the submaximal stimulus was consistent with the fact that neuromuscular junctions are present along the whole length of the muscle. Action potentials initiated at the end of the muscle distant from the recording electrodes would be expected to take more than 30 msec to arrive at the electrodes (distance 22 mm, average speed of conduction 0·70 m/sec) and such action potentials may be seen to occur (Fig. 6*a*). On maximal nerve stimulation (Fig. 6*b*), however, the response was not longer in duration than the response to direct stimulation (Fig. 6*c*). Furthermore, if the electrodes were suitably placed, the peak amplitude of the response to maximal nerve stimulation was little smaller than the amplitude of the action potential evoked by direct stimulation. If one electrode was kept fixed and the other moved along the surface of the muscle there was only a small variation in the latency and time course of the response, the latency being least in the central part of the muscle, in which the nerves were seen to enter, and increasing towards each end of the muscle (Fig. 7). A simple way of explaining these results (cf. Katz & Kuffler, 1941) is to suppose that action potentials arise almost but not quite simultaneously at many neuromuscular junctions along each fibre of the muscle, the variation in latency being due to different conduction times along the motor axons to the neuromuscular junctions at different points along the muscle. In each fibre, then, after propagating through only a short distance, the action

potential collides with and abolishes the action potential from a neighbouring junction.

In some preparations isolated single maximal stimuli applied to the nerve gave rise to action potentials of irregular amplitude and duration. If, however, the stimuli followed a period of repetitive stimulation (for example, 100/sec for 30 sec), after a short interval the response became regular in amplitude and shorter in duration. In these preparations facilitation was apparently required at some of the junctions before action potentials were initiated at them (cf. Brown & Harvey, 1938; Hutter, 1952; Liley & North, 1953).

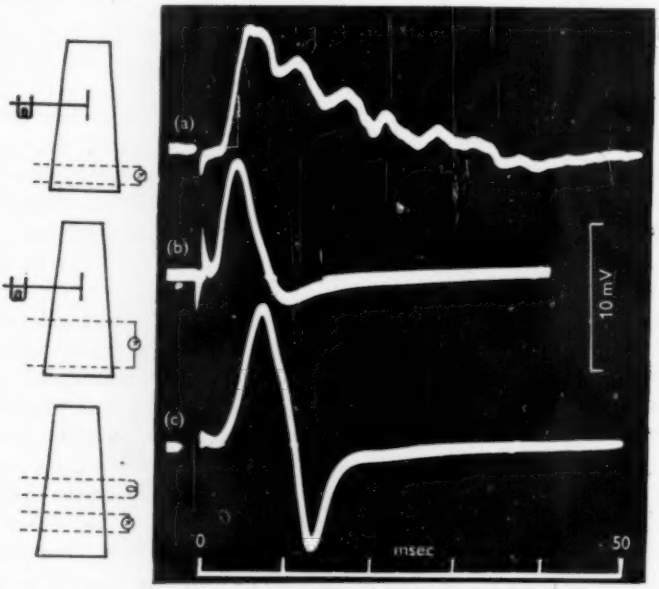

Fig. 6. Action potentials recorded externally from an anterior latissimus dorsi muscle 20-day-old chick. Muscle length, 28 mm. Temperature, 36° C. (*a*) Response to submaximal stimulation of nerve; recording electrodes 2 and 6 mm from central end of muscle; voltage calibration 1 mV. (*b*) Response to maximal nerve stimulation; recording electrodes 2 to 10 mm from 'central' end of muscle. (*c*) Response to direct stimulation (neuromuscular transmission blocked with 8×10^{-5} M tubocurarine Cl); recording electrodes 6 and 10 mm, stimulating 14 and 18 mm from 'central' end of the muscle. Voltage calibration (*b*) and (*c*) 10 mV.

Two tests of this explanation have been made, (*a*) by curarizing part of the muscle and (*b*) by cutting the nerve supply to part of the muscle.

Effect of curarizing part of the muscle. Figure 8 illustrates the effect on the action potential of blocking neuromuscular transmission in part of the muscle. In the 'curarized' region the latency of the action potential increases by the time necessary to propagate from neuromuscular junctions in the 'uncurarized' part of the muscle. In the 'uncurarized' part of the

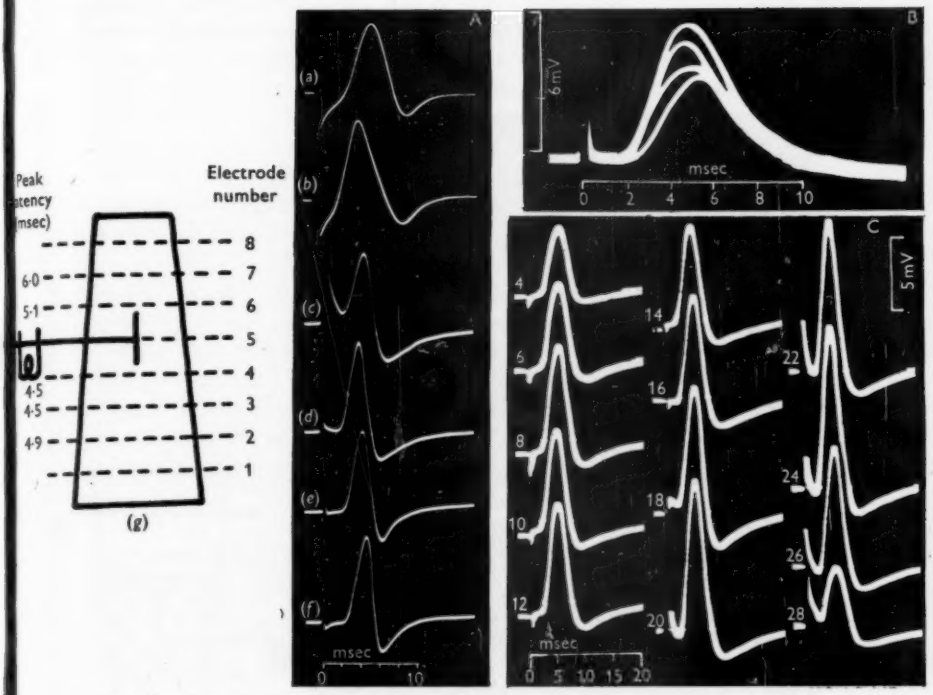

Fig. 7. Action potentials recorded externally from three anterior latissimi dorsi, *A*, *B*, *C*, in response to maximal nerve stimulation. *A*: 22-day-old chick; muscle length, 30 mm; temperature 33° C. Muscle mounted on multi-electrode assembly (*g*), electrodes 4 mm apart. Action potentials recorded between electrodes **7**, 8(*a*); **6**, 8(*b*); **5**, 1(*c*); **4**, 1(*d*); **3**, 1(*e*); **2**, 1(*f*); upward deflexion denotes negativity of electrode with bold number. Interval between first peak of response and stimulus artifact at different points along muscle indicated below in (*g*). *B*: 22-day-old chick; muscle length, 24 mm; temperature, 36° C; superimposed sweeps; indifferent electrode 2 mm from 'central' end of muscle which was treated with 132 mM-$MgCl_2$ to make responses monophasic (Niedergerke, 1956). Distance between electrodes in order of increasing amplitude of responses: 6, 8, 10, 12 mm; average speed of conduction subsequently determined after curarization, 0·5 m/sec. *C*: 28-day-old chick; muscle length, 34 mm; temperature, 35° C, d.c. recording. Indifferent agar wick electrode at 'central' end of muscle; second electrode, fluid surface. Distances in mm between fluid surface and indifferent electrode indicated by number at the side of each response.

muscle only minor changes occur in the action potential in spite of the curarization of the remaining two thirds of the muscle. These changes are probably accounted for by the diffusion of the tubocurarine applied to the lower part of the muscle into the 'uncurarized' region and the resulting blockade of the more susceptible junctions.

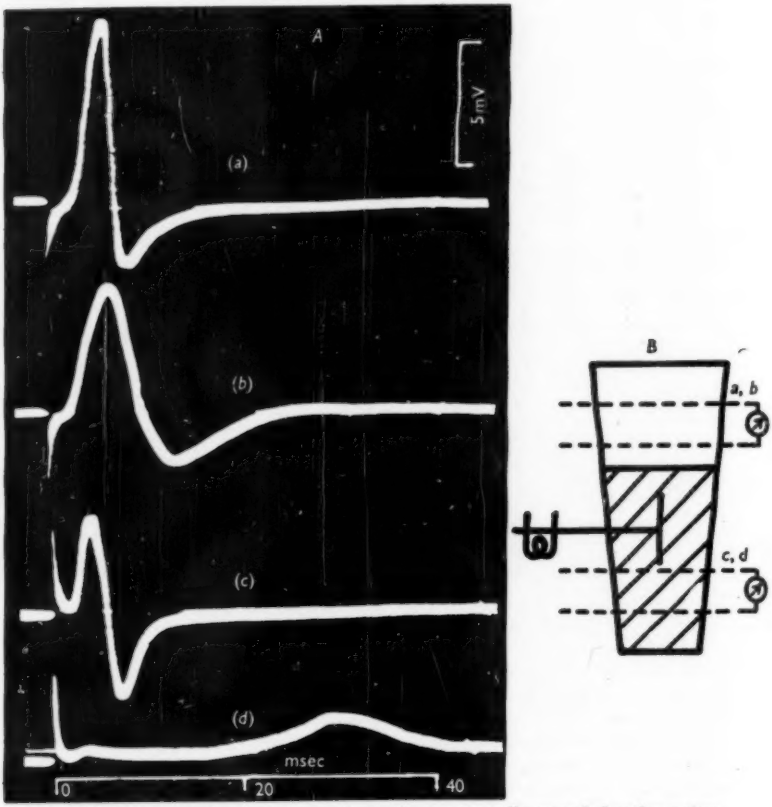

Fig. 8. Effect of curarization of part of muscle on externally recorded action potential from an anterior latissimus dorsi muscle: same preparation as Fig. 7*C*. Agar wick electrodes, d.c. recordings. (*a*) and (*c*) recorded (with electrodes in position shown in *B*) before curarization. (*b*) and (*d*) recorded from same positions after the part of the muscle shown shaded in *B* had been bathed in 5×10^{-5} M tubocurarine Cl for 7 min. Upward deflexion denotes negativity of electrode nearer the centre of the muscle.

Effect of cutting part of nerve supply. The nerve to the anterior latissimus dorsi branches extensively before entering the muscle; it is therefore possible by cutting the appropriate branches to denervate acutely part of

the muscle. The effect of this procedure on the indirectly evoked action potential is illustrated in Fig. 9. In the part of the muscle to which the nerve supply was 'intact' (Fig. 9*A a, d*) there were only minor changes in the time course and amplitude of the response. It is likely that these changes occurred because some muscle fibres had been totally denervated. In the 'denervated' region, the latency of the response was increased by the time taken for action potentials to propagate along the muscle from the most proximal junctions which remained innervated. The small reduction in the amplitude of the action potential in the 'intact' region, after cutting more than half the branches of the nerve to the muscle, shows that at least a majority of fibres in the muscle must be innervated by several axons. This result is also clear from the experiment illustrated in Fig. 9*B*. The action potential in the 'denervated' region evoked by nerve stimulation, again after cutting more than half the branches of the nerve, is very little smaller than the action potential evoked by direct stimulation of the whole muscle. The response of most of those fibres which extended between the position of the stimulating and recording electrodes must therefore have survived 'denervation' at least at one neuromuscular junction.

Mechanical recording

Response to nerve stimulation. Figure 10*A* illustrates the responses of three varieties of anterior latissimus dorsi muscles and shows that these muscles are capable of producing twitches to single maximal stimuli applied to the nerve. The anterior latissimus dorsi of the chick and the latissimus dorsi of the pigeon have both been shown directly to be made up largely or exclusively of multiply-innervated fibres and it may be inferred that this is also the case for the anterior latissimus dorsi of the rook (see Ginsborg & Mackay, 1961). It does not seem likely that the twitch was due to a small number of focally innervated fibres which have resisted detection histologically; the twitch in such fibres, as exemplified by the twitch of the posterior latissimus dorsi (a muscle which usually contains few, if any, multiply-innervated fibres (Ginsborg & Mackay, 1961) has a much more rapid time course (Fig. 10*B*). This is not conclusive evidence, however, since there may be differences in the speed of the twitch in different focally innervated fibres of the bird equivalent to the differences in speed between 'fast' and 'slow' mammalian muscle fibres (see, for example, Buller, Eccles & Eccles, 1960).

On repetitive stimulation of the nerve at minimum frequencies, varying from preparation to preparation between 15 and 35/sec, the anterior latissimus dorsi muscle responded with maximal sustained tension throughout the period of stimulation (up to 3 min). This behaviour was in contrast to that of the posterior latissimus dorsi. The tetanic tension (produced by

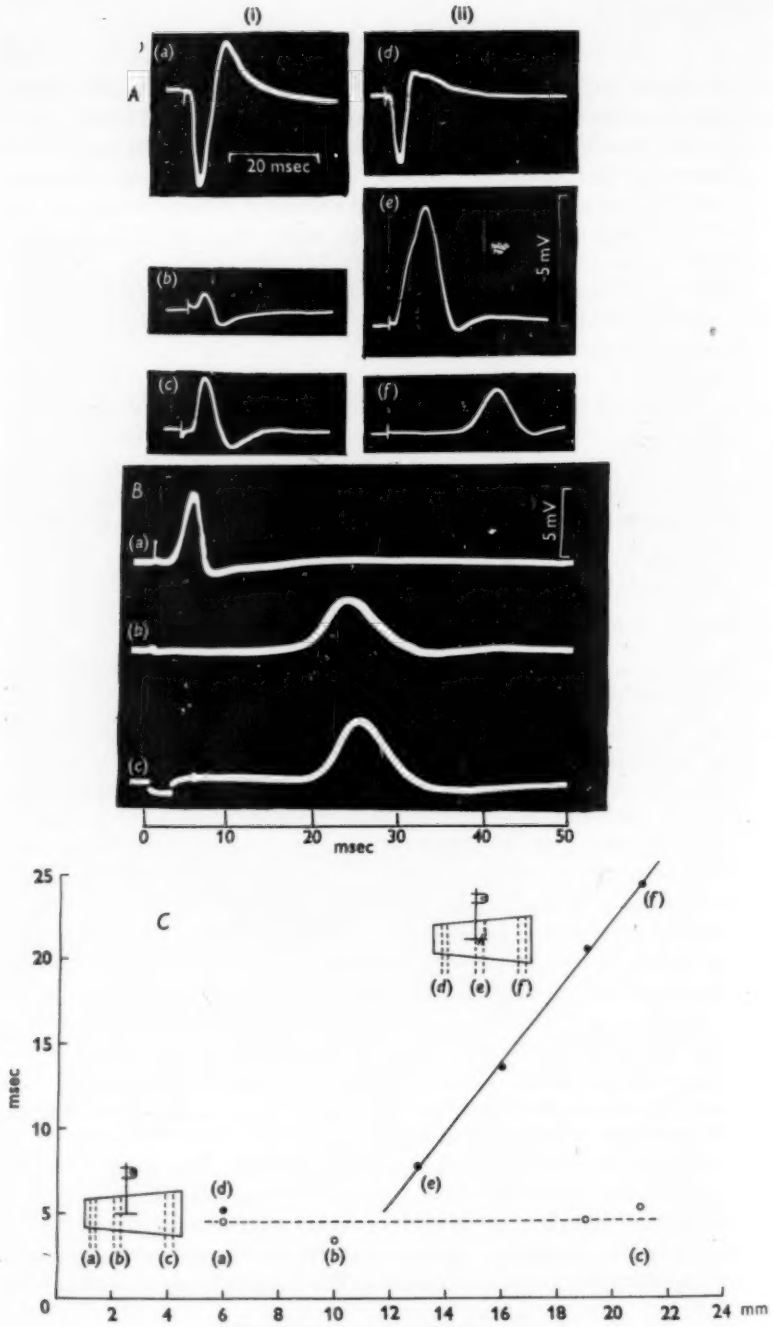

Fig. 9. For legend see facing page.

repetitive nerve stimulation at a minimum frequency in excess of 100/sec) in this muscle declined to less than half its maximum value in about 25 sec.

Response to depolarizing agents. When drugs known to cause depolarization at the motor end-plate (decamethonium, succinylcholine and carbaminoylcholine) were added to the fluid bathing the anterior latissimi dorsi, a prolonged increase in tension of the muscle resulted (Figs. 11 and

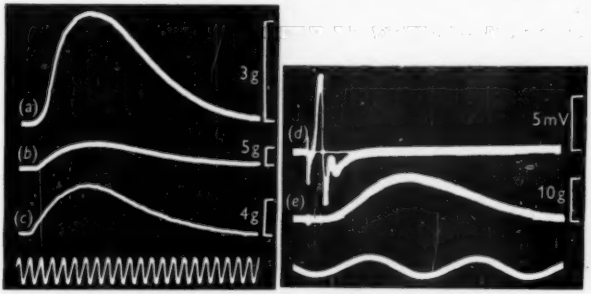

Fig. 10. 'Isometric twitches' in response to nerve stimulation. (*a*), anterior latissimus dorsi of chick; (*b*), anterior latissimus dorsi of rook; (*c*) latissimus dorsi of pigeon; (*e*), posterior latissimus dorsi of chick. The action potential (*d*) and twitch (*e*) were recorded simultaneously. Time marker, 20 msec. 37° C.

12*a*). The amplitude of the maximal isometric tension (a maximal effect was caused by decamethonium iodide (C_{10}), for example, at a concentration of $1{\cdot}7 \times 10^{-6}$ M in all three varieties of muscle tested) was almost identical with that produced by tetanic stimulation of the nerve; this result suggests that all the fibres in these muscles were taking part in the response. Contractures of similar amplitude but of longer duration were produced by bathing the muscle in a solution enriched in potassium. In

Fig. 9. Effect of 'denervating' part of muscle on externally recorded action potential. *A*: 23-day-old chick; muscle length 24 mm; temperature 29° C. (i) Nerve intact, (ii) branches of nerve to 'central' half of muscle cut. Recording electrodes 2 mm apart. Distance of the inner recording electrode from peripheral end of muscle indicated in *C* by letters affixed to points on graph which correspond to lettering of records. Upward deflexion in (*b*), (*c*), (*e*) and (*f*) and downward deflexion in (*a*) and (*d*) denotes negativity of inner electrode. Maximal nerve stimulation. *B*: 21-day-old chick; muscle length 26 mm; temperature 36° C. Recording electrodes 2 and 6 mm from 'central' end of muscle. Upward deflexion, negativity of inner electrode. (*a*) Nerve intact, maximal nerve stimulation; (*b*) branches to 'central' half of muscle cut, maximal nerve stimulation; (*c*) direct stimulation of curarized muscle: stimulating cathode 12 mm from inner recording electrode. *C*: variation in 'peak' latency at different points along normal (○) and partly denervated (●) muscle. Preparation of Fig. 9*A*. Abscissa, distance between inner recording electrode and peripheral end of muscle (mm). Ordinate, interval between first peak of action potential and stimulus artifact (msec).

seven experiments on anterior latissimi dorsi of the chick, the time taken for the tension to decay from the maximum to half the maximum value varied between 3 and 8 min in the presence of $1{\cdot}7 \times 10^{-6}$ M C_{10}, whereas this time varied between 9 and 15 min in the presence of 100 mM-KCl (replacing NaCl). In the rook (Fig. 12) the difference in duration was even

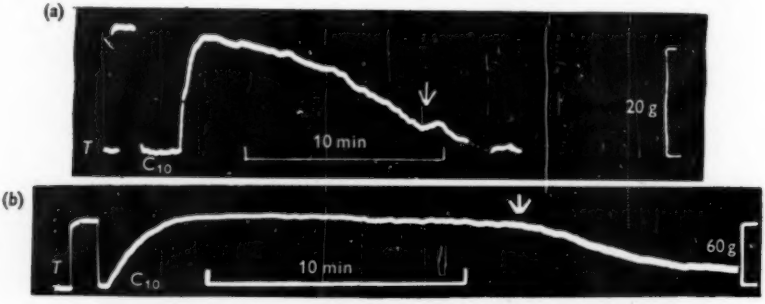

Fig. 11. Comparison between isometric tension produced by repetitive nerve stimulation at 40/sec, T, and by the addition of decamethonium iodide to the bath to give a final concentration of $1{\cdot}7 \times 10^{-6}$ M. (*a*) anterior latissimus dorsi of chick, (*b*) latissimus dorsi of pigeon. At arrows, bathing solution drained and replaced with Krebs–Henseleit solution. 37° C.

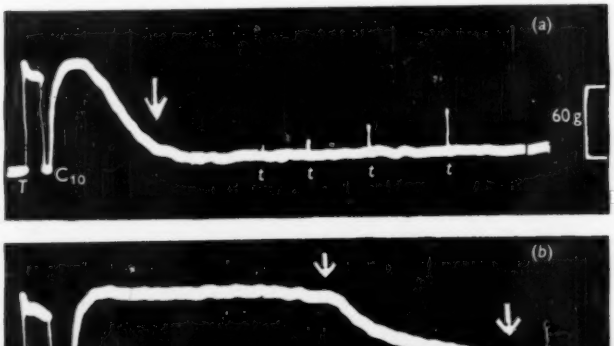

Fig. 12. Anterior latissimus dorsi of rook. Comparison between isometric tension produced (*a*) by decamethonium iodide $1{\cdot}7 \times 10^{-6}$ M, and that produced (*b*) by replacing 100 mM-NaCl with 100 mM-KCl. Common time and tension scales in (*a*) and (*b*). T, tetanic tension produced by nerve stimulation at 40/sec. At arrows, bath drained and refilled with Krebs–Henseleit solution. Recovery of neuromuscular transmission delayed after the contracture had subsided, as may be seen from the responses to the 5 sec test tetani, t, applied to the nerve. 37° C.

more pronounced. The shorter duration of the 'decamethonium contracture' probably reflects the relatively rapid decline of the depolarization of the membrane due to 'desensitization' (see for example, Thesleff, 1958; Fatt, 1950).

As a rule prolonged contractures could not be elicited from the posterior latissimus dorsi. In one out of sixteen experiments, however, a contracture of similar time course to that obtained in the anterior latissimus dorsi did occur; the maximum tension produced, 2 g, was, however, only about one tenth of the tetanic tension the muscle was able to develop. Since on histochemical staining of posterior latissimi dorsi a small band of fibres with multiple neuromuscular junctions was, on one occasion, observed (Ginsborg & Mackay, 1961), it seems possible that the anomalous response was due to the presence of such fibres in the muscle in question. Two other muscles which failed to give prolonged contracture in two experiments were the extensor carpi radialis profundus and the extensor digiti III and IV. No multiply-innervated fibres have been detected in these two muscles (Ginsborg, 1960; Ginsborg & Mackay, 1961).

DISCUSSION

It is evident that avian muscle fibres with multiple neuromuscular junctions are similar in certain ways to the slow fibres of the frog (Kuffler & Vaughan Williams, 1953*a*, *b*). Both types of fibre are innervated at numerous points by several axons and it seems probable, also, that both types of fibre are distinguished histologically from focally innervated fibres in another way, namely in having a 'Felderstruktur' as compared to a 'Fibrillenstruktur' (see Krüger, for example, 1952; Krüger & Günther, 1956). (For evidence relating to the frog, see Kuffler & Vaughan Williams, 1953*b*; for the bird, Ginsborg & Mackay, 1961). Both types of fibre respond to prolonged depolarization with prolonged contracture, a property which is not shared with focally innervated fibres of either the bird or frog (see, for example, Taylor, 1953; Kuffler & Vaughan Williams, 1953*b*; Horowicz & Hodgkin, 1956). On the other hand, one clear difference between the multiply-innervated fibres of bird and the slow fibres of the frog is the ability of the fibres of the bird to produce propagated action potentials; in this respect the fibres of the bird resemble certain multiply-innervated intrafusal fibres in the frog (Koketsu & Nishi, 1957*a*) and some crustacean muscles (Fatt & Katz, 1953).

Although it seems probable that in the bird and the frog multiple innervation, 'Felderstruktur', and the ability to produce prolonged contractures are all properties of the same variety of muscle fibre, the connexion between these properties is obscure. There is clearly no immediate connexion between type of innervation and ability to produce prolonged contracture;

however, evidence is accumulating which suggests that the innervation of a muscle fibre does in some unknown way affect both structural (Krüger & Günther, 1957) and functional (Buller *et al.* 1960) characteristics of the myoplasm. Nor can it be supposed that a 'Felderstruktur' as such confers the ability to produce prolonged contractures. Thus although 'Felderstruktur' fibres exist in the mammal (Krüger, 1952), Rückert (1930, 1930/1) found that the application of constant currents, which evoke prolonged contractures both in slow fibres of the frog and in multiply-innervated fibres of the bird, failed to do so in mammalian skeletal muscles, with the exception of certain foetal muscles and the diaphragm of the white mouse. The particular type of fine structure of the myoplasm which gives rise to the characteristic 'Felderstruktur' appearance of the cross-section of the fibre may be necessary, but it is apparently not sufficient to allow a fibre to undergo a prolonged contracture.

SUMMARY

1. Single skeletal muscle fibres, with multiple neuromuscular junctions, of the biventer cervicis of the chick have been investigated with microelectrodes. The results obtained were confirmed by those resulting from external recording from the anterior latissimus dorsi, which is made up largely or exclusively of fibres with multiple neuromuscular junctions.

2. These fibres are innervated by several motor axons, and respond to direct and to maximal indirect stimulation with propagated action potentials. In the fibres of the anterior latissimus dorsi action potentials on maximal indirect stimulation were initiated at many of the junctions on each fibre.

3. The mechanical response to 'depolarizing agents' of the anterior latissimus dorsi of the chick and the rook and of the latissimus dorsi of the pigeon was a prolonged contracture: the tension of the maximal contracture was approximately equal to the maximal isometric tetanic tension produced by nerve stimulation. As a rule the posterior latissimus dorsi of the chick did not respond with prolonged contractures; this muscle usually contains few if any fibres with a dense innervation.

I should like to thank Professor W. L. M. Perry for his continued hospitality and encouragement and Mr W. T. S. Austin and Mr N. E. Condon for their unfailing help. I should also like to thank Dr J. G. Blackman for preparing Fig. 1.

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ENZYMIC OXIDATION OF 5-HYDROXYTRYPTAMINE BY PIG SERUM

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A study of the inhibitor and substrate specificities of the amine oxidases in mammalian blood plasma has shown that these enzymes are distinct from the intracellular amine oxidase. The substrates of the plasma oxidases in the living animal are unknown.

In an earlier study dialysed pig serum was used as source of enzyme (Blaschko, Friedman, Hawes & Nilsson, 1959). This preparation, as well as a precipitate obtained from the serum by 45–55 % saturation with ammonium sulphate, oxidized 5-hydroxytryptamine (5-HT) as well as many other amines. Benzylamine was one of the substrates most rapidly oxidized, and the provisional name 'benzylamine oxidase' was therefore proposed for this enzyme (Bergeret, Blaschko & Hawes, 1957).

The purpose of this paper is to describe an experiment which shows that 5-HT is not a substrate of the benzylamine oxidase of pig plasma. This finding is the outcome of a further purification of the plasma enzyme.

METHODS

Pig serum was obtained from the slaughterhouse and purified as described below.

Enzymic activity was measured manometrically at 37·5° C. In the experiment to be described in detail the gas phase was oxygen in the flasks containing benzylamine (10^{-2} M); in those containing 5-HT ($0{\cdot}5 \times 10^{-2}$ M) and paraphenylenediamine (PPD; 10^{-2} M) it was air. The pH of the buffers used was 6·0; with benzylamine and 5-HT the buffer was 0·067 M sodium phosphate, with PPD it was 0·01 M sodium acetate. These conditions were chosen after preliminary experiments to find optimal conditions for the reactions studied.

The enzyme unit (e.u.) was defined as the amount of enzyme that caused the uptake of 1 μl. O_2 in 30 min.

Copper was determined in the trichloracetic acid extract according to Gubler, Lahey, Ashenbrucker, Cartwright & Wintrobe (1952).

Purification of pig serum fractions. The procedure adopted closely followed that of Holmberg & Laurell (1948), but their second extraction with the chloroform–ethanol mixture was omitted.

Following the Swedish authors, the material precipitated by between 35 and 50 % saturation with $(NH_4)_2SO_4$ was obtained. The precipitate was taken up in tap water, dialysed for 4 hr against running tap water and then during the night against distilled water. After dialysis the pH was adjusted to 5·5 by adding dilute acetic acid (5–10 % (v/v)).

After centrifugation at 5500 rev/min for 15 min, the supernatant was decanted and

retained. In the experiment of Table 1, 242 ml. of supernatant was obtained from 1520 ml. of serum. Of this, 7 ml. was used for the manometric assay (fraction 1 of Table 1): to the remainder ethanol was added at 0° C, to a final concentration of 15% (v/v). After centrifugation for 15 min at 5500 rev/min, both the sediment, taken up in a little saline solution, and the supernatant, were dialysed during the night against running tap water; the volume of the sediment (fraction 2 of Table 1) was 65 ml., that of the supernatant (fraction 3 of Table 1) was 260 ml. Of the sediment, 4 ml. was removed for enzyme assay. To the remainder, after adjusting the pH to 6·5 with dilute acetic acid, a mixture of chloroform–ethanol (1:9 (v/v)) was added to a final concentration of 50% (v/v). A clear supernatant fluid was removed by centrifugation at 5500 rev/min for 30 min. The precipitate was extracted six times with 20–25 ml. of saline solution. The extracts, which had an intensely blue colour, were combined and concentrated by precipitation with $(NH_4)_2SO_4$ to a saturation of 65% (v/v). This precipitate was dialysed against running tap water, followed by distilled water; the final volume of this fraction was 17·5 ml. (fraction 4 of Table 1). The copper content of the final extract was 23·6 μg Cu/ml.

All centrifugations were carried out at 0° C.

RESULTS

Pig plasma contains, in addition to benzylamine oxidase, a polyphenol oxidase (Holmberg & Laurell, 1948; Kolb, 1957), known as caeruloplasmin, a copper-containing protein of blue colour. In the experiments of Blaschko *et al.* (1959) it had been noted that the ammonium sulphate

TABLE 1. Oxidation of 5-hydroxytryptamine, benzylamine and paraphenylenediamine by fractions obtained from pig serum; activities expressed in enzyme units

Fraction / Substrate	PPD	Benzylamine	5-HT	Ratio PPD: 5-HT
No. 1; 35–50% ammonium sulphate precipitate	180,000	16,500	4,360	41·2
No. 2; 15% ethanol precipitate	161,000	3,050	4,030	39·9
No. 3; 15% ethanol supernatant	13,700	16,400	0	—
No. 4; final extract	89,000	0	2,420	36·7

precipitates rich in benzylamine oxidase activity had a blue colour. It was therefore decided to apply the procedure for the purification of caeruloplasmin in order to find out whether or not the amine oxidase activity remained with the caeruloplasmin.

The results of these experiments are shown in Table 1. It can be seen that the ammonium sulphate precipitate oxidized all three substances used as substrates. The ethanol precipitate had little activity with benzylamine as substrate, but it contained most of the catalyst acting on 5-HT and PPD. On the other hand, practically all the benzylamine oxidase activity was recovered in the supernatant fluid from the ethanol precipitation; this fraction had little activity with PPD and none with 5-HT. In the final extract (fraction 4) about one half of the activity on both PPD and 5-HT was retained, but there was no benzylamine oxidase activity. The experiment was repeated several times, with essentially similar results.

DISCUSSION

The experiment just described shows that the oxidation of 5-HT is not brought about by the benzylamine oxidase present, but by caeruloplasmin. This conclusion is supported by the observation that the ratio (rate of oxidation of PPD):(rate of oxidation of 5-HT) remained fairly constant during the purification procedure.

PPD is generally used as a typical substrate of caeruloplasmin. That caeruloplasmin oxidizes 5-HT has been reported by several authors (Porter, Titus, Sanders & Smith, 1957; Martin, Eriksen & Benditt, 1958; Curzon & Vallet, 1960). We have since shown that caeruloplasmin acts also on hydroxyindoles substituted in positions 4, 6 or 7 (Blaschko & Levine, 1960). It is not known whether these reactions are of importance for the biological inactivation of 5-HT and other hydroxyindoles.

For our knowledge of the plasma amine oxidases our finding is of interest, because it eliminates benzylamine oxidase as a possible agent in the biological inactivation of 5-HT. The biological significance of benzylamine oxidase remains obscure.

SUMMARY

The oxidation of 5-hydroxytryptamine (5-HT) by pig serum has been studied. Fractionation of the serum proteins has shown that 5-HT is not a substrate of the amine oxidase present in the serum; its oxidation is catalysed by caeruloplasmin, the copper-containing protein of plasma.

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A STUDY OF SOME ELECTROPHYSIOLOGICAL PROPERTIES OF HUMAN INTERCOSTAL MUSCLE

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It is of interest to compare the electrophysiological properties of normal human skeletal muscle with those of other mammalian species. In the present investigation the spontaneous subthreshold activity at the myoneural junction has been studied, and the membrane constants of the muscle fibres have been determined. As did Dillon, Fields, Gumas, Jenden & Taylor (1955); and Creese, Dillon, Marshall, Sabawala, Schneider, Taylor & Zinn (1957), we also found that human intercostal muscles proved to be excellent preparations for studies *in vitro*. It will be shown that the electrophysiological properties of these muscles are rather similar to those of other mammalian species, except that the frequency of the miniature end-plate potentials (m.e.p.p.) is considerably lower, and the membrane resistance higher.

METHODS

During thoracotomy operations on eight patients of both sexes and with no known muscle disease, specimens of external intercostal muscle were removed from the 5th, 6th, or 7th intercostal space in the mid-axillary line. The muscles were carefully dissected so that the specimens contained both origin and insertion, with continuous periosteum. Immediately upon removal the specimens were placed in continuously oxygenated Tyrode solution and taken to the laboratory for dissection and study. As described by Creese *et al.* (1957), longitudinal fusiform bundles of fibres, with their fibrous origin and insertion intact, could be dissected under a binocular microscope. The isolated bundles were about 15 mm long and 0·2–0·5 mm in diameter. They were mounted in a bath kept constantly at 37° C.

The composition of the bathing fluid was that used by Liley (1956) and it was oxygenated by bubbling 95 % O_2 + 5 % CO_2 through it immediately before its introduction into the muscle bath. The bath held about 30 ml. of solution, which was changed continuously at a rate of about 500 ml./hr.

The usual techniques were employed for intracellular recording with capillary glass micro-electrodes (Fatt & Katz, 1951). Electrodes of between 5 and 10 MΩ resistance were used, and the input time constant of the recording circuit was 20–50 μsec. M.e.p.p.s were photographed from an oscilloscope tube on single sweeps of 50 msec/cm. Usually at least twenty m.e.p.p.s were recorded from each fibre.

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Membrane constants were determined by the method of 'square-pulse analysis' (Hodgkin & Rushton, 1946; Fatt & Katz, 1951). The duration of the current pulse was 100–120 msec. The experimental procedure and methods of calculations were similar to those used by Boyd & Martin (1959) with the cat tenuissimus muscle.

RESULTS

It was possible to maintain the isolated muscle bundle in good condition for several hours with no significant decay of resting potentials, which ranged from 70 to 90 mV, with a mean of about 80 mV (Table 1). At the

TABLE 1. The mean values and their S.E. for resting membrane potential, amplitude and frequency of m.e.p.p.s in four human intercostal muscles. For comparison similar values obtained from cat tenuissimus and rat diaphragm are shown. The numbers in brackets indicate the number of muscle fibres

Type of muscle	Resting membrane potential (mV)	Amplitude of m.e.p.p.s (mV)	Frequency of m.e.p.p.s (per sec)
Human intercostal	80·9 ± 1·00 (15)	0·74 ± 0·013 (26)	0·26 ± 0·065 (25)
Cat tenuissimus (Boyd & Martin, 1956)	75	0·54 ± 0·14 (54)	1·43 ± 0·88 (72)
Rat diaphragm (Liley, 1956)	73	0·6	1–2

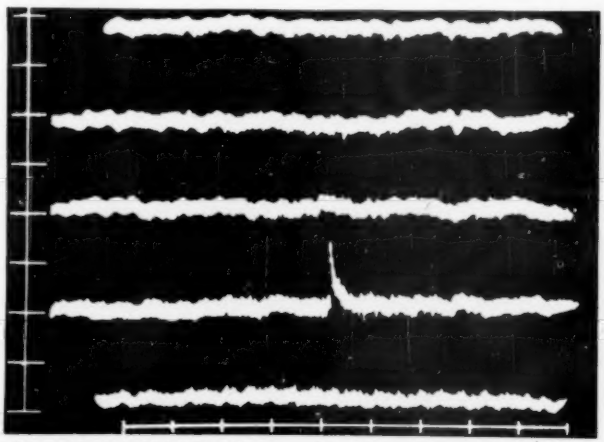

Fig. 1. A m.e.p.p. recorded on single successive sweeps. Human intercostal muscle; male aged 65. Time marker, 50 msec; voltage calibration, 0·5 mV.

neuromuscular junction spontaneous subthreshold electrical activity was recorded (Fig. 1). These m.e.p.p.s were similar to those observed in frog muscle (Fatt & Katz, 1952), in rat diaphragm (Liley, 1956) and in cat tenuissimus muscle (Boyd & Martin, 1956). Their mean amplitude was about 0·7 mV, and they appeared to occur at random intervals. The

frequency of discharge was, however, considerably lower than that reported from other species, the average being only 0·2/sec (Table 1). An increase in the osmotic pressure of the bathing fluid resulted in an immediate and marked increase of the frequency of the m.e.p.p.s (Fig. 2) (cf. Liley, 1956).

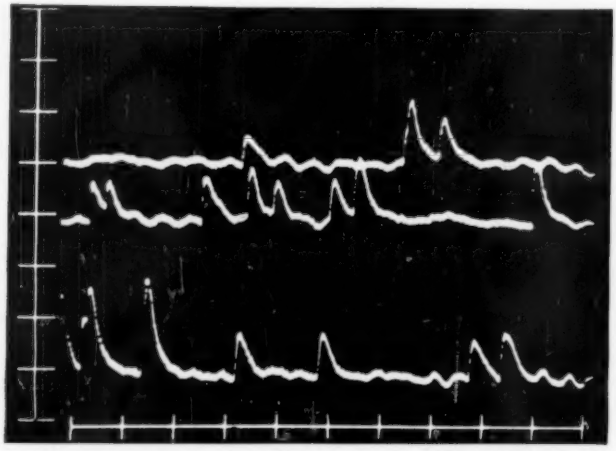

Fig. 2. Increased rate of m.e.p.p.s produced by hypertonic Tyrode solution. Human intercostal muscle; female aged 43. Time marker, 10 msec; voltage calibration, 0·5 mV.

The membrane constants were determined by inserting two microelectrodes into the fibre, at first as close together as possible, and then again at distances of about 0·5 and 1·0 mm between the electrodes. A rectangular hyperpolarizing current pulse was passed through one of the electrodes and the resultant change in membrane potential was recorded by the other. By applying the cable theory of Hodgkin & Rushton (1946) the potential change, V, produced by a steady current, I, through the membrane is given by the equation,

$$V = \tfrac{1}{2}I\sqrt{(r_m . r_1)}\exp\left[-x/\sqrt{(r_m/r_1)}\right],$$

where x is the electrode separation, r_m the transverse resistance of a unit length of fibre membrane, and r_1 the internal longitudinal resistance per unit length of fibre. The term $\sqrt{(r_m . r_1)}$ is the space constant λ of the fibre.

When the ratio $V:I$ was plotted on a logarithmic scale against the electrode separation, almost straight lines (Fig. 3) were obtained. λ is the distance over which the electrotonic potential fell to 1/e of its value. $\frac{1}{2}\sqrt{(r_m . r_1)}$ was obtained from the point at which the lines intersected the vertical axis. From these two values r_m and r_1 were calculated.

The specific resistivity of mammalian myoplasm, R_1, has been estimated as 125 Ω.cm by Boyd & Martin (1959), and this value was also used in our calculations. The fibre radius, ρ, was obtained from the relation

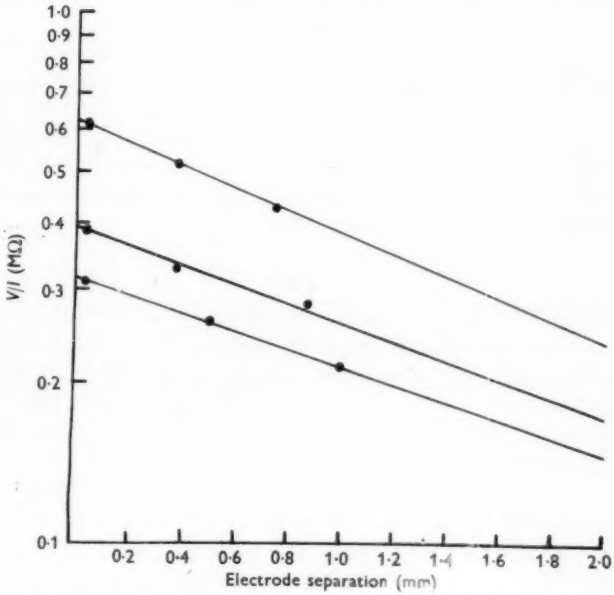

Fig. 3. Spatial decay of electrotonic potentials in three fibres. Ordinate, amplitude of electrotonic potential, when a steady state is recorded, divided by amplitude of current pulse; logarithmic scale. Abscissa, separation between current and voltage-recording electrodes.

TABLE 2. Data derived from two human intercostal muscles in which curves were obtained similar to those in Fig. 3. *RP* = resting membrane potential at the beginning and end of each experiment

	Fibre	$\frac{1}{2}\sqrt{(r_m . r_1)}$ (Ω)	$\lambda = \sqrt{(r_m/r_1)}$ (mm)	τ_m (msec)	ρ (μ)	R_m (Ω cm²)	C_m (μF/cm²)	*RP* (mV)
Female, 30 years	1	6×10^5	1·75	20·5	24	3200	6·4	84–82
	2	$3{\cdot}9 \times 10^5$	2·10	20·5	33	3420	6·0	85–71
	3	$3{\cdot}9 \times 10^5$	2·44	20·5	35	4200	4·9	88–78
Male, 38 years	1	$6{\cdot}4 \times 10^5$	2·35	18·0	27	5100	3·5	86–72
	2	$6{\cdot}1 \times 10^5$	2·27	15·5	27	4700	3·3	90–80
	3	$3{\cdot}1 \times 10^5$	2·65	14·0	41	4200	3·3	86–81
	4	$4{\cdot}3 \times 10^5$	2·15	23·0	32	3700	6·2	83–70
Mean			2·24	18·9	31	4070	4·8	
Cat's tenuissimus (Boyd & Martin, 1959)								
Mean			1·10	4·9	22	1430	3·5	

$\rho = \sqrt{(R_1/\pi r_1)}$ and the transverse resistance of a unit area of membrane, R_m, by $R_m = 2\pi\rho r_m$. The time constant of the membrane, τ_m, was measured from the time taken for the potential to rise to 83 % of its maximum steady value, at about zero electrode separation. The membrane capacitance per unit area, C_m, was then derived from $C_m = \tau_m/R_m$.

The results of such calculations for seven fibres are shown in Table 2, along with the corresponding values for the cat tenuissimus muscle, for comparison. It is evident that the membrane resistance of the human intercostal muscle is considerably higher than that of the cat tenuissimus muscle. The calculated values for the fibre diameters agree with the value of about 60μ obtained by direct measurements from histological transverse sections by Creese *et al.* (1957).

DISCUSSION

The isolated human intercostal muscle has proved to be a stable preparation which is well suited for studies with intracellular electrodes. It can be obtained during thoracotomy procedures or by biopsy. Thus it seems appropriate to use that muscle in studies of the alterations in the electrophysiologic behaviour of human skeletal muscle in disease of muscle and lower motor neurone.

The low frequency of m.e.p.p.s in the human as compared with other species is difficult to explain. It is possibly related to the fact that all our specimens were obtained from adult patients, i.e. from mammals of a much greater age than that of any laboratory animal. Or it may be that a low 'miniature' frequency is a unique property of the intercostal muscle, as opposed to other skeletal muscles. The transverse resistance of the fibre membrane is about 4000 Ω.cm^2, which is higher than that of the cat tenuissimus muscle, but about the same as that observed by Fatt & Katz (1951) for frog muscle.

SUMMARY

1. By the use of intracellular electrodes the spontaneous miniature end-plate potentials and the membrane constants of the muscle fibre have been studied in the isolated human intercostal muscle.

2. The mean resting membrane potential was about 80 mV. The average frequency of miniature end-plate potentials was about 0·2/sec, and their mean amplitude, 0·7 mV.

3. The transverse resistance of the fibre membrane was about 4000 Ω.cm^2 and the membrane capacitance varied between 3 and 6μF/cm^2.

We are indebted to Drs O. Dahlbäck and E. Linder for providing the muscle specimens. This investigation was aided by United States Public Health Service research grant B-2646 from the National Institute of Neurological Diseases and Blindness, by the Muscular

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THE SUPPORT OF ACTIVE POTASSIUM TRANSPORT IN HUMAN RED CELLS BY NUCLEOSIDES AND DEOXYNUCLEOSIDES

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Glucose is the predominant substrate for glycolysis in human red cells and the ultimate source of energy for the active transport of ions across the cell membrane. Lowy, Jaffe, Vanderhoff, Crook & London (1958) showed that lactic acid may also be produced from several nucleosides, and other workers have shown that these compounds support the accumulation of potassium (Gabrio, Donohue & Finch, 1955; Prankerd, 1956; Kahn & Cohen, 1957). A restoration of active cation transport is needed when cold-stored cells are incubated at 37° C, in order to prevent the entry of sodium chloride into the cells and eventual lysis owing to the colloid osmotic pressure of haemoglobin (Davson & Danielli, 1938). A study has therefore been made of the efficacy of several nucleosides and deoxynucleosides in supporting both lactic acid production and potassium uptake in red cells of cold-stored blood.

METHODS

The preparation and incubation of cells have been described (Whittam, 1960).

Extracts of the cells and of the cell suspension were made in trichloroacetic acid, and ribose was estimated by the method of Ceriotti (1955), deoxyribose by the method of Burton (1956), and lactate by the method of Barker & Summerson (1941). Potassium was estimated with a flame photometer (Amoore, Parsons & Werkheiser, 1958). Measurements of potassium influx were made by means of ^{42}KCl, following the procedure of Glynn (1956). To measure the influx on the addition of a nucleoside, ^{42}KCl solution was added simultaneously and the ^{42}K in the cells was assayed after incubation for 15 and 30 min. Although an instantaneous measurement of influx is impossible, the effects found in this way are referred to as immediate changes in influx.

RESULTS

Lactic acid production from nucleosides

The production of lactic acid from nucleosides and deoxynucleosides described by Lowy *et al.* (1958) has also been found in the present experiments. Table 1 shows the results of two experiments in which more lactate was formed from the nucleosides than from the corresponding deoxynucleosides. Thus, adenosine produced 3·0 and deoxyadenosine 1·9 m-

mole/l. cells/hr. The ribosides and deoxyribosides of guanine and hypoxanthine produced about 20 % less lactate than those of adenine, but about 50 % more lactate was produced from the nucleoside than from the deoxynucleoside. No lactate was produced from glucose.

In order to determine the number of moles of lactic acid produced per mole of sugar metabolized, the loss of ribose and deoxyribose was measured. The methods used for the sugar estimations did not distinguish between the free sugar and the sugar in the form of an ester or a nucleoside, and the column in Table 1 showing the loss of ribose or deoxyribose represents the difference between the total amount of sugar before (20·0 m-mole/l. cells) and after incubation. The loss of ribose varied from 3·9 m-mole/l. cells/hr with inosine to 4·5 m-moles/l. cells/hr with adenosine. The molar ratio of the lactate produced:sugar metabolized shows that less than one mole of

TABLE 1. The production of lactate from nucleosides and deoxynucleosides

Substance added	Lactate produced (m-mole/l. cells/hr)	Loss of ribose or deoxyribose (m-mole/l. cells/hr)	Ratio, moles of lactate formed:moles of sugar lost
Guanosine	2·2	4·2	0·5
Adenosine	3·0	4·5	0·7
Inosine	2·3	3·9	0·6
Deoxyguanosine	1·4	4·0	0·4
Deoxyadenosine	1·9	4·0	0·5
Deoxyinosine	1·6	2·6	0·6
Glucose	0	—	—

Conditions as in Table 2.

lactate was produced for each mole of ribose that was metabolized, the ratio being 0·6 for inosine, 0·7 for adenosine and 0·5 for guanosine. Comparable figures for lactate produced per mole of deoxyribose metabolized, are 0·6 mole for deoxyinosine, 0·5 mole for deoxyadenosine and 0·4 mole for deoxyguanosine. The results show that less than 1 mole of lactate was formed from each mole of sugar metabolized, both with nucleosides and deoxynucleosides; complete conversion to lactate would give ratios of 1·6, and part of the sugars must have produced other compounds, such as hexose phosphates and 2:3-diphosphoglyceric acid (Prankerd, 1956).

Potassium movements and nucleosides

Preliminary experiments showed that inosine and adenosine can replace glucose as the energy source for active potassium transport both in fresh red cells and in cells previously stored at 4° C with glucose for 5 days. In order to study the effect of glucose and nucleosides in cells that had been further depleted of potassium, red cells from 4-weeks-old cold-stored blood were washed free from glucose and allowed to stand a further day at 4° C

in the saline medium before incubation at 37° C. Measurements were made of the potassium influx at the start of incubation at 37° C and after 2 hr. The net changes in potassium concentration after incubation for 3 or 4 hr were also measured.

Potassium influx. The main result was that the potassium influx after the addition of 10 mM glucose was the same as that in control cells without glucose (about 1 m-mole/l. cells/hr), both at the start of incubation and after 2 hr (Table 2). The addition (in concentrations of 10 mM) of ribose, deoxyribose, glucose plus adenine or hypoxanthine, or the pyrimidine ribosides, cytidine, thymidine and uridine, also had no significant effect on

TABLE 2. The influx and net movements of potassium in red cells at 37° C after previous cold storage at 4° C (m-mole/l. cells/hr)

Additions to cells	Time when influx measured (hr) 0	2	Net change in concentration	Active net transport	Active part of influx at 2 hr	Ratio, net active transport: active part of influx
None	1·0 ± 0·1 (10)	0·9	−1·6	0	0	—
Glucose	1·2 ± 0·2 (5)	0·8	−1·5	0·1	0	—
Inosine	2·9 ± 0·3 (9)	3·0	+0·8	2·4	2·1	1·1
Adenosine	2·9	3·8	+1·5	3·1	2·9	1·1
Guanosine	2·3	3·2	+1·0	2·6	2·3	1·1
Deoxyinosine	2·0	—	−0·6	1·0	—	—
Deoxyadenosine	2·3	4·1	+1·5	3·1	3·2	1·0
Deoxyguanosine	1·8	2·8	+0·5	2·1	1·9	1·1

Red cells from 4-weeks old cold-stored blood were washed free from glucose and left for a further day at 4° C in phosphate saline medium. The suspension (10% haematocrit, pH 7·4) was then shaken in air at 37° C and additions were made to give final concentrations of 10 mM for glucose and 5 mM for ribosides and deoxyribosides. Mean values of at least two determinations of the influx and the net change in concentration are given: for 5 or more determinations (no. of observations in parentheses) the S.E. is also given.

the potassium influx. In contrast, the addition of inosine, adenosine or guanosine caused an immediate increase in the potassium influx from 1·0 to 2·9, 2·9 and 2·3 m-mole/l. cells/hr, respectively, even at the start of incubation. After incubation for 2 hr the values were 30–40% greater with adenosine and guanosine and unchanged with inosine. An immediate increase in the potassium influx was also found on the addition of deoxyinosine, deoxyadenosine and deoxyguanosine, the values being 2·0, 2·3 and 1·8 m-mole/l. cells/hr respectively (Table 2); these values are 20–30% lower than those found after the addition of the analogous nucleoside.

Net changes in potassium concentration. Table 2 shows the rate of the net change in the potassium concentration during incubation for 3–4 hr. The rate of active net transport is taken to be the rate of net change with a compound added, minus the rate of net change without any addition. The addition of any of the nucleosides or deoxynucleosides supported an active net transport of potassium of 1·0–3·1 m-mole/l. cells/hr. These values may

be compared with the active component of the potassium influx after 2 hr incubation. (The active potassium influx is the influx with a compound added less the influx in the cells without any addition.) The rate of active net transport and the active influx are within 10 % of each other, as shown by the values of 1·0 and 1·1 for the ratio of the active net transport : active part of the influx.

The active part of the influx was abolished when additions were made to the cell suspension of either 1 mM Na iodoacetate or 1 μM ouabain, which inhibit glycolysis and active cation transport respectively (Schatzmann, 1953). The results show that an active inward movement of potassium occurred into cold-stored red cells when various nucleosides or deoxynucleosides were added.

DISCUSSION

Previous work has shown that various nucleosides support an accumulation of potassium by human red cells under conditions when glucose is also effective (Gabrio *et al.* 1955; Prankerd, 1956; Kahn & Cohen, 1957), and the main finding described in this paper is that lactic acid production and active potassium movements may also be supported by various nucleosides and deoxynucleosides under conditions when glucose is ineffective. The simplest explanation for the lack of effect of glucose is that cold-stored cells become so depleted of adenosine triphosphate (ATP) that glucose 6-phosphate is no longer formed from glucose, and lactic acid is therefore not produced. Erythrocytes are known to lose ATP during cold storage, although the precise time when its concentration would be too low to react with glucose via hexokinase does not seem to have been determined.

The effects of nucleosides and deoxynucleosides are probably a result of the production of pentose phosphates by phosphorolysis (Gabrio & Huennekens, 1955), which can be degraded to lactic acid without the need of ATP for the formation of sugar phosphate. The metabolism of the pentose phosphates to lactic acid will be accompanied by a resynthesis of ATP from the adenosine diphosphate and adenylic acid which accumulate during cold storage (Whittam, 1958). ATP is probably the link between glycolysis and active potassium transport in human red cells and must be rapidly resynthesized in order to explain the immediate effect of the nucleosides and deoxynucleosides in increasing the potassium influx. Overgaard-Hansen (1957) showed that inosine, guanosine and adenosine caused a resynthesis of ATP of 0·16 m-mole/l. cells/min, which would be fast enough to account for the immediate effects of these compounds on potassium transport.

SUMMARY

1. Human red cells from 4-weeks-old cold-stored blood were washed free from glucose and stored for a further day at 4° C without glucose. The lactic acid production and potassium transport were measured during subsequent incubation at 37° C after the addition of glucose or various nucleosides.

2. Incubation with glucose produced a negligible amount of lactic acid and the loss of cell potassium was equal to that found in control cells without glucose. The addition of inosine, adenosine or guanosine or of the corresponding deoxynucleosides caused a production of lactic acid (1·4–3·0 m-mole/l. cells/hr) and about 0·5 mole lactic acid (0·4–0·7) was produced for each mole of ribose or deoxyribose that was metabolized.

3. The nucleosides and deoxynucleosides supported an active inward movement of potassium, shown by measurements both of the influx and the net changes in the cell potassium concentration.

4. It is suggested that the support of an active uptake of potassium by nucleosides and deoxynucleosides under conditions in which glucose is ineffective is due to the formation of pentose phosphates, which yield lactic acid.

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THE HIGH PERMEABILITY OF HUMAN RED CELLS TO ADENINE AND HYPOXANTHINE AND THEIR RIBOSIDES

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Human red blood cells are highly permeable to glucose, which is their normal substrate for glycolysis. Several nucleosides also yield lactic acid, however, and like glucose they support the active transport of sodium and potassium ions (Kahn & Cohen, 1957; Lowy, Jaffe, Vanderhoff, Crook & London, 1958). During an investigation of the effects of nucleosides on cation transport a number of questions arose about the permeability of the red-cell membrane to these compounds (Whittam, 1960). This paper describes the resulting study of the uptake of inosine, adenosine, hypoxanthine and adenine by human red cells. The main findings are the high membrane permeability to these compounds and the intracellular conversion of adenine to a non-diffusible form when a substrate for glycolysis was provided.

METHODS

Preparation of cells for incubation. Blood which had been stored for 4 weeks at 4° C in the 'acid-citrate dextrose' solution used in Blood Transfusion Centres (the following solution is mixed with 420 ml. of blood: anhydrous glucose 3·0 g, disodium hydrogen citrate hydrated 2·0 g, water 120 ml.) was obtained from the Radcliffe Infirmary, Oxford. The cells were washed five times with saline medium (mM: NaCl 150, KCl 5, sodium phosphate, pH 7·4, 10) to remove glucose, and the white cells and the upper layer of red cells were removed by suction. The remaining red cells were suspended in the saline medium (50% haematocrit) and shaken in air in stoppered conical flasks in a water-bath at 5 or 37° C. Samples of the suspension were removed after various times for chemical analysis. Precautions to ensure sterile conditions of incubation were not taken, but it is unlikely that bacterial growth during the longest period of incubation of 2 hr would affect the results.

Haematocrit determinations. Samples of the suspension were taken at the beginning of each incubation for haematocrit determinations, which were made by centrifuging the suspension in capillary tubing of uniform bore (13 cm long and 3 mm bore) for 30 min at 1500 *g*. The haematocrit value was taken to represent the actual cell volume, since the volume of trapped fluid under these conditions is probably not more than 2% (Jackson & Nutt, 1951).

Chromatography

Samples of the medium after various periods of incubation were dried on Whatman No. 1 filter paper in a stream of air at room temperature after previously washing the paper according to the directions of Eggleston & Hems (1952). A separation of inosine and adenosine from hypoxanthine and adenine respectively was required by using a solvent which

itself did not absorb in ultra-violet light. The most convenient solvent was found to be 0·1 M sodium borate, pH 10·0 (see Burke, 1954, for electrophoretic separation), with which an ascending solvent front for 6 hr at 20° C gave separations with the R_F values shown in Table 1. The separations of the compounds were reproducible and tailing was not encountered. Inosine was eluted from the paper with 4·0 ml. 0·1 M sodium phosphate (pH 3·0) and estimated from its absorption at 250 mμ, using an extinction coefficient of 11,800 (Kalckar, 1947).

Radioactive chemicals

8-^{14}C adenine sulphate and 8-^{14}C hypoxanthine were supplied by the Radiochemical Centre, Amersham, and standard solutions of these were added to the saline medium. The fall in the radioactivity in the medium was measured by drying known volumes (20–80 μl.) of the medium on Whatman No. 1 filter paper, which was then placed below an end-window G–M tube for the assay of the disintegration rate. A standard solution of the labelled purine, with respect both to radioactivity and to concentration, gave counts per minute proportional to the volume of the solution dried on the paper, and, therefore, allowed the concentration in samples of medium to be calculated from the disintegration rate. 1 μc gave 65,000 counts/min under the present conditions of counting.

TABLE 1. R_F values of purine-containing compounds after 6 hr ascending migration at 20° C

Compound	R_F	Compound	R_F
Inosine	0·86	Hypoxanthine	0·64
Xanthosine	0·85	Deoxyadenosine	0·53
Deoxyinosine	0·79	Uric acid	0·53
Guanosine	0·78	Guanine	0·43
Deoxyguanosine	0·67	Adenine	0·37
Adenosine	0·64		

Sodium borate solution (0·1 M, pH 10·0), in a glass tank (52 cm deep), was allowed to ascend Whatman No. 1 filter paper on which solutions of the compounds had been dried and which was supported from the lid of the tank. The chromatograms were then dried at room temperature and examined under a low-pressure mercury lamp (Chromatolite, Hanovia, Slough), when the compounds were located as dark areas on a light background. Photographs of the chromatograms under ultra-violet light were also made, essentially as described by Markham & Smith (1949).

RESULTS

The permeability of red cells to nucleosides and deoxynucleosides

Inosine. The uptake of inosine by human red cells was taken to be equal to the disappearance of inosine from the saline medium of a 50% cell suspension. Inosine was added to several portions of the suspension to give concentrations in the medium of 36, 18 and 9 mM in order to see in what way the uptake of inosine by the cells was proportional to the initial external concentration. Samples of the suspension were centrifuged after being shaken for 5, 10 and 20 min at 37° C, and inosine was analysed in the medium. The results (Table 2) show that already after 5 min the concentrations in the medium had fallen to 47, 58 and 68% of the initial concentrations, when the latter were 36, 18 and 9 mM respectively. Little further change occurred between 5 and 20 min. The percentage uptake by the

cells (68 %) with the lowest concentration in the medium (9 mM) was greater than that (47 %) when the concentration was 36 mM. Since a limited amount of phosphorolysis of inosine will lower the intracellular concentration of inosine, this result is to be expected in order to produce the same distribution of inosine between cells and medium with both low and high concentrations. The results are consistent with an uptake of inosine by the cells sufficient to produce approximately equal concentrations in the cells and medium.

TABLE 2. The fall in the inosine concentration in the medium during the incubation of red cells at 37° C

Time (min)	Inosine concn. in medium (mM)	Percentage fall in inosine concn. in medium	Minimum rate of uptake of inosine by cells (m-mole/l. cells/hr)
0	36·0	0	200
5	19·0	47	
10	21·5	40	—
20	18·0	50	—
0	18·0	0	120
5	7·5	58	
10	8·0	55	—
20	7·5	58	—
0	9·0	0	70
5	3·0	68	
10	3·0	68	—
20	2·0	78	—

Washed, cold-stored red cells were added to samples of saline medium containing inosine at concentrations of 9, 18 and 36 mM. The suspensions (50 % haematocrit) were shaken in conical flasks in a water-bath at 37° C and samples were removed after 5, 10 and 20 min. The inosine concentration in the medium was measured after centrifugation to remove the cells.

The rates of net entry of inosine calculated from these results are minimum ones, because inosine may have reached equilibrium in less than 5 min. In view of the limitations of the technique of centrifugation for the sampling of the medium for short periods of incubation, it did not seem worth while to attempt to measure the exact rate of uptake. However, the minimum values for the rate of entry are 200, 120 and 70 m-mole/l. cells/hr for initial concentrations in the medium of 36, 18 and 9 mM respectively. These results show that, within this range of concentration, the minimum rate of net entry of inosine into the cells was directly proportional to the concentration in the medium.

Adenosine. Accurate measurement of the rate of uptake of adenosine is complicated, because this compound is deaminated to inosine in human red cells (Conway & Cooke, 1939), and two processes in series will, therefore, determine the rate of uptake of adenosine from the medium. The uptake

will be governed first by the permeability and secondly by the rate of lowering of the intracellular concentration owing to the deamination to inosine. To obtain an estimate of the uptake of adenosine by cells the absorption spectrum of the medium was measured after different periods of incubation when the initial concentration of adenosine in the medium was 20 mM. The fall in the height of the peak for adenosine at 260 mμ

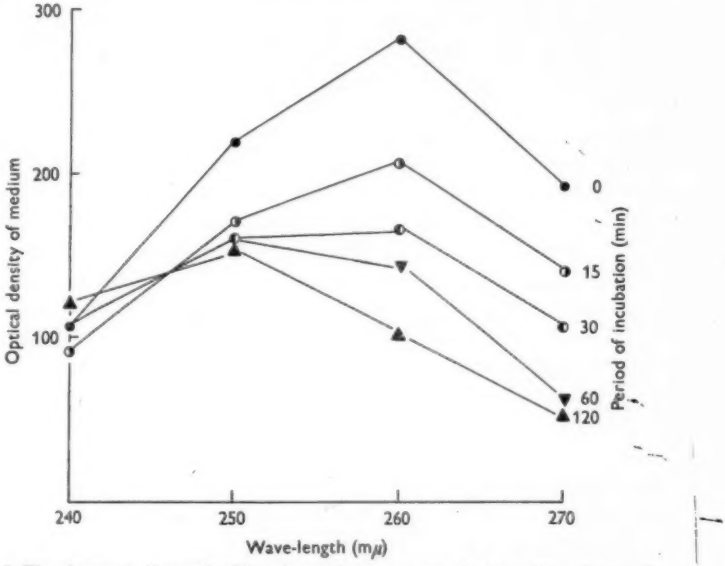

Fig. 1. The change in the peak of the absorption spectrum of the medium during the incubation of red cells with adenosine. Washed, cold-stored red cells were incubated at 37° C in a saline medium containing 20 mM adenosine. The cells were separated from the medium after various periods of incubation and the absorption spectrum of the medium in the range 240–270 mμ was measured.

within 15 min shows that adenosine was taken up from the medium (Fig. 1), and the minimum rate of uptake was 110 m-mole/l. cells/hr. Furthermore, the peak of absorption gradually changed from 260 to 250 mμ until, after 2 hr, the curve was characteristic of hypoxanthine or inosine to which adenosine is deaminated. This result shows that there is a rapid entry of adenosine into human red cells.

The permeability of red cells to hypoxanthine and adenine

In view of the high permeability of human red cells to inosine and adenosine it was of interest to see whether the purines hypoxanthine and adenine also readily crossed the cell membranes. In the following experi-

ments, the disappearance of either ^{14}C-labelled hypoxanthine or adenine was followed from the medium of a cell suspension with an approximately 50 % haematocrit.

Hypoxanthine. Preliminary experiments showed that the hypoxanthine concentration in the medium fell to a constant level within 5 min of the addition of washed cells and that no further change occurred after incubation for 1 hr. A variation of the initial hypoxanthine concentration from 0·2 to 6·8 mM made no difference to the percentage of the medium hypoxanthine which entered the cells. Table 3 shows that about 50 % (range 51–57) of the hypoxanthine initially present in the medium was taken up by the cells, and that the ratio of the concentrations in cells: medium must consequently have been between 1·0 and 1·3.

To see whether glycolysing cells took up more hypoxanthine than washed cells, guanosine was added as a convenient substrate for lactic acid production (Whittam, 1960). Table 3 shows that the addition of guanosine had no effect on the amount of hypoxanthine entering red cells, which was about 50 % (range 46–52) of the initial amount present. It may be concluded that hypoxanthine reaches equilibrium across the red cell membrane within 5 min and that no appreciable concentration gradient was established between the cells and medium.

The fall in the concentration of hypoxanthine in the medium was also measured after incubation at 5° C and after the addition separately at 37° C of 0·4 mM uric acid, 3·8 mM allantoin and 7·0 mM adenine, as it was thought that these compounds might inhibit the uptake of hypoxanthine by the cells owing to their similar molecular structure. Within the limits of the present techniques, these conditions of incubation had no effect on the extent of the uptake of hypoxanthine.

Adenine. The fall in the adenine concentration in the medium was measured when the range of the initial concentration was 1·6–7·7 mM. Inosine was added to part of the suspension as a substrate for glycolysis to see whether it caused a greater uptake of adenine. Table 3 shows that after incubation at 37° C for 30–60 min, the fall in the adenine concentration in the medium without inosine was between 53 and 63 %, which indicates that adenine must have reached approximately equal concentrations in the cells and medium. 53 % of the adenine in the medium was also absorbed by the cells incubated with inosine when the initial adenine concentration was 7·7 mM, but at lower concentrations of adenine a greater percentage was removed. Thus, when the initial concentrations were 0·6–1·6 mM more than 95 % of the adenine in the medium was taken up by the cells in the presence of inosine. More than 95 % of the adenine in the medium was also taken up by the cells during incubation with either glucose, deoxyinosine or adenosine. It therefore seems that cold-stored

red cells provided with a substrate for glycolysis absorb more than 95% of the adenine in the medium when its concentration is 1·6 mM or lower. The uptake of 52–65% by glycolysing cells with the higher concentrations of adenine (3·0, 4·6 and 7·0 mM) suggests that the greater uptake of 95% at the lower concentrations was not the result of an active transport of adenine into the cells that depends on glycolysis, because an active uptake would be expected to operate over the whole range of concentration.

TABLE 3. The fall in the concentrations of hypoxanthine or adenine in the medium during the incubation of red cells at 37° C

Purine	Additions to suspension	Initial concn. in medium (mM)	Percentage fall in concn. in medium after 30–60 min
Hypoxanthine	None	6·8	51
		3·4	57
		2·2	55
		0·5	52
	Guanosine 10 mM	6·8	47
		3·4	52
		2·2	46
		0·5	50
Adenine	None	7·7	53
		4·6	56
		3·0	53
		1·6	63
	Inosine 10 mM	7·0	52
		4·6	64
		3·0	65
		1·6	> 95
		0·65	> 95
	Inosine 10 mM + iodoacetate 1 mM	0·59	63
	Glucose 10 mM	0·65	> 95
	Deoxyinosine 10 mM	0·55	> 95
	Adenosine 10 mM	0·66	> 95

Washed, cold-stored red cells were added to samples of saline medium containing either ^{14}C-labelled hypoxanthine or ^{14}C-labelled adenine at the concentrations shown. Further additions of various compounds were made to the suspensions (50% haematocrit) before they were incubated at 37° C for 30–60 min. After the separation of the cells by centrifugation the concentration of hypoxanthine or adenine in the medium was determined by the measurement of the decay of ^{14}C from Whatman No. 1 filter paper on which small volumes (0·02–0·08 ml.) of the medium had been dried.

The time course of the uptake of adenine both at 5 and 37° C is shown in Fig. 2. Equilibrium was reached after 5 min at each temperature, but only 67% of the adenine in the medium was taken up by the cells at 5° C, in contrast to 97% uptake at 37° C. The effect of lowering the temperature, therefore, was to prevent the extra uptake of adenine by the cells with inosine that was found during incubation at 37° C. Figure 2 shows that after a portion of the suspension was transferred from 5 to 37° C, an additional 30% of the adenine in the medium was taken up by the cells.

The converse experiment of transferring a sample of suspension from 37 to 5° C had no effect on the adenine concentration in the medium, suggesting that the adenine taken up at 37° C was not able to leak back into the medium on cooling.

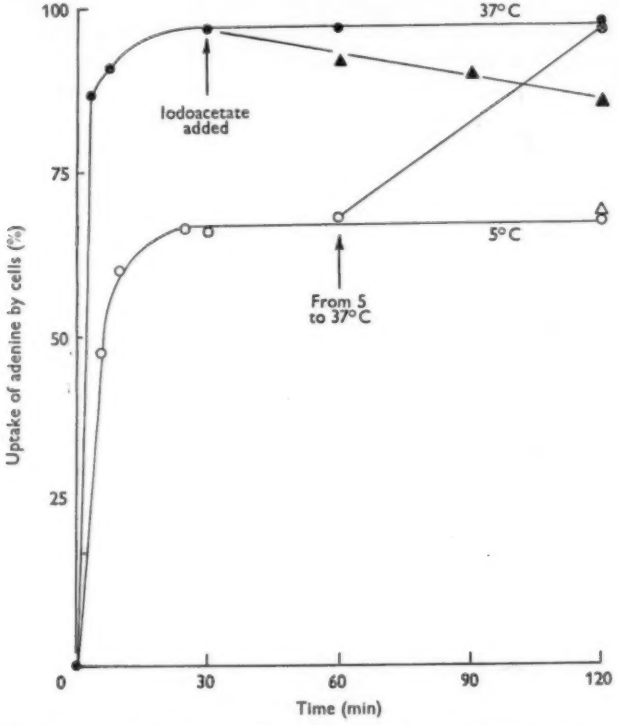

Fig. 2. The percentage uptake of adenine from the medium during the incubation of red cells at 5 or 37° C. Washed, cold-stored red cells were incubated either at 5 (○) or at 37° C (●) in a saline medium (50 % haematocrit) containing 20 mM inosine and about 0·6 mM ^{14}C adenine. The uptake of adenine by the cells was followed by measuring the fall in ^{14}C-adenine in the medium, from which the cells were separated by centrifugation. The decay of ^{14}C was measured from Whatman No. 1 filter paper on which samples of the medium (0·02–0·08 ml.) had been dried. 1 mM sodium iodoacetate was added to portions of the suspension either at the start of incubation (△) or after 30 min (▲). A sample of the suspension was transferred after 60 min from 5 to 37° C and left for a further 60 min (⊗).

The avid uptake of adenine at 37° C in the presence of a substrate for glycolysis suggested that iodoacetate, by its inhibition of glycolysis (triose-phosphate dehydrogenase), might counteract the effect of the substrate. This proved to be so (Table 3 and Fig. 2), for the uptake was 63 % instead

of 97 % after the addition of 1 mM iodoacetate. This compound was also added after 30 min incubation with inosine, and Fig. 2 shows that only a small amount (12 %) of the adenine initially taken up leaked from the cells into the medium.

DISCUSSION

Inosine and adenosine

The permeability of human red cells to nucleosides and purines does not appear to have been previously studied, and precise measurements of the rates of movement have not been possible in the present work owing to the high permeability that was found. The results with inosine and adenosine, however, show that these compounds were taken up by the cells from the medium and that the equilibrium of inosine between cells and medium in approximately equal concentrations occurred within 5 min. Because of the deamination of adenosine in human red cells (Conway & Cooke, 1939), adenosine did not reach a steady distribution across the membrane, but its uptake during the first 15 min of incubation indicated a high membrane permeability. Although only a qualitative description of the permeability is possible, the present study of the uptake of inosine and adenosine has shown that it is high enough not to be a limiting factor when these substances are used as sources of energy by human erythrocytes, which have been previously stored for 4 weeks at 4° C. The high membrane permeability to these compounds is consistent with their utilization as sources of energy to give the immediate increase in potassium influx which was found on their addition to cold-stored cells (Whittam, 1960).

Hypoxanthine and adenine

Hypoxanthine reached a roughly equal distribution between cells and medium within 5 min when it was initially present in the medium. No evidence was found for an active transport of hypoxanthine either into or out of the cells, for neither the temperature of incubation (5 or 37° C), the presence or absence of glycolysis nor the addition of compounds of a similar molecular structure like uric acid, adenine and allantoin had any effect on the extent of the uptake.

The uptake of adenine resembled that of hypoxanthine in being fast, but differed in being greater when a substrate for glycolysis was present at low concentrations of adenine in the medium. Several compounds that are converted to lactic acid by red cells (inosine, glucose, deoxyinosine and adenosine) caused an almost complete uptake of adenine from the medium (more than 95 %) when the initial concentration was 1·6 mM or lower. When glycolysis was suppressed, either by deprivation of substrate, by cooling to 5° C or by the addition of iodoacetate, the cells in a 50 % suspension took up only about 60 % of the adenine of the medium. Once

adenine had been taken up by the cells at 37° C, the addition of iodoacetate caused only a small leakage, and subsequent cooling to 5° C caused no release of adenine to the medium.

The results suggest that glycolysing human red cells may convert adenine into a form that is unable to pass readily out of the cell. At high concentrations of adenine (3–7 mM), the percentage of adenine taken up by the cells was the same both in the presence and absence of glycolysis, presumably because the amount of adenine that may be converted to a non-diffusible form was low relative to the absolute amount present. This observation makes it unlikely that adenine was actively transported into the cells. The most likely compounds that could arise from adenine and be unable to leave the cells are the adenine nucleotides, and it is of interest that Nakao, Nakao, Tatibana, Yoshikawa & Abe (1959) briefly described a synthesis of adenosine triphosphate by human erythrocytes, which has also been observed under the conditions of the present experiments (J. S. Wiley & R. Whittam, unpublished). Possible differences in purine metabolism that may explain the greater uptake of adenine than hypoxanthine by glycolysing cells are being investigated.

SUMMARY

1. A study has been made of the uptake of inosine, adenosine, hypoxanthine and adenine from a saline medium during the incubation of cold-stored human red cells under various conditions.

2. The inosine concentration in the medium fell within 5 min at 37° C to a constant level that was consistent with an even distribution of inosine between the cells and medium. Adenosine was quickly taken up by red cells and was deaminated to inosine.

3. Hypoxanthine reached an approximately even distribution between cells and medium within 5 min at 37° C; this was not changed by either cooling or the addition of guanosine as a substrate for glycolysis.

4. At concentrations of adenine in the medium of 3·0–7·7 mM, approximately equal concentrations between cells and medium were reached within 5 min at 37° C. At low concentrations (0·6–1·6 mM) adenine was almost completely taken up by the cells in the presence of substrates for glycolysis; cooling and iodoacetate abolished this metabolically dependent uptake. It was concluded that adenine may be converted into another form to which the membrane is impermeable.

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PROCEEDINGS OF
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LUND UNIVERSITY MEETING

29–30 *July* 1960

The recording of the membrane potential in skeletal muscle by the sucrose-gap method. By D. Elmqvist. *Department of Pharmacology, University of Lund, Sweden*

Secretion from the submaxillary gland after partial post-ganglionic denervation. By N. Emmelin. *Institute of Physiology, University of Lund, Sweden*

One to three days after section of the auriculo-temporal nerve in the cat the parotid gland shows periods of secretory activity, alternating with periods of rest. When the gland is secreting, the flow of saliva occurs in bursts, and this type of secretion has been termed 'paroxysmal secretion'. It is supposed to be due to acetylcholine, released in bursts from the endings of the degenerating post-ganglionic nerve fibres (Emmelin & Strömblad, 1958).

Attempts were made to see whether a similar secretion can be evoked in the submaxillary gland of the cat. Owing to the fact that many of the synapses of the parasympathetic pathway are situated within this gland, a complete post-ganglionic parasympathetic denervation cannot be made; but it is possible to make a partial denervation by tracing the chorda tympani, under the dissecting microscope, along the salivary duct and cutting it close to the gland (Emmelin, 1960). In such preparations under chloralose anaesthesia no secretion could be detected 1–2 days after the operation. This failure could be due to the fact that the denervation was partial only, the amounts of acetylcholine released being too small to stimulate the secretory cells. In further experiments these cells were therefore sensitized by previous section of the preganglionic parasympathetic fibres. About 3 weeks later the degenerated chorda was dissected towards the hilum of the gland. These operations were carried out under ether and hexobarbitone anaesthesia, respectively. On the following day the cat was given chloralose and the submaxillary ducts were cannulated. Saliva was found to flow more or less continuously from the gland of the operated side, but not from the contralateral gland. The flow continued

after the intravenous injection of hexamethonium or dihydroergotamine. It was accelerated by eserine and abolished by a small dose of atropine.

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The late Professor Thunberg's micro-respirometer and barospirator. By P. PETERSEN. *Institute of Physiology, University of Lund, Sweden*

Thunberg appears to have been the first to discover that nervous tissue respires, takes up oxygen and gives up CO_2. This discovery he made with his micro-respirometer by which he could measure oxygen consumption and CO_2 production in small units of tissue (1904). Using this apparatus he could also demonstrate that traces of certain metals, for example manganese salts, strongly catalyse tissue respiration.

In 1926 Thunberg published the principle of his barospirator which technically was elaborated at the Physiological Institute in Lund by Enghoff. Thunberg introduced a new principle, ventilation of the lungs without respiratory movements. This is achieved by enclosing the patient in a closed respiratory chamber in which the air pressure is made to pulsate with a suitable amplitude and frequency. The barospirator was primarily used in the treatment of poliomyelitis, but Thunberg's principle has since been employed in the U.S.A. for immobilization of the lungs in the treatment of pulmonary tuberculosis.

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Instruments and appliances recently designed and made at the Institute of Physiology, University of Lund, Sweden. By B. CLEMENTZ. *Institute of Physiology, University of Lund, Sweden*

Retardation of rapid growth in plant tissues (yellow peas) by the enzyme inhibitor semicarbazide. By G. KAHLSON and ELSA ROSENGREN. *Institute of Physiology, University of Lund, Sweden*

Rapid mammalian tissue growth, such as embryonic development and reparative growth in healing skin wounds, is associated with a high histamine-forming capacity in embryonic and granulation tissues. Embryonic

development is prevented (Kahlson & Rosengren, 1959) and the healing of wounds retarded (Kahlson, Nilsson, Rosengren & Zederfeldt, 1960) by semicarbazide, which inhibits the enzyme histidine decarboxylase. The growth of roots and leaves in germinating peas is also conspicuously retarded by semicarbazide in concentrations down to 10^{-5}M.

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Effects of androgens and oestrogens on mammary glands of hypophysectomized rats injected with thyroxine, insulin and cortisone. By DORA JACOBSOHN. *Institute of Physiology, University of Lund, Sweden*

The growth reaction of the mammary glands to ovarian hormones fails to occur in hypophysectomized rats. However, injection of hypophysectomized rats with insulin and cortisone with or without thyroxine in addition to ovarian hormones did result in mammary growth. This effect was assumed to be due to the beneficial actions of thyroxine, insulin and cortisone on the general metabolism.

Androgens do not produce growth of alveolar lobules in rats without pituitary gland. Instead, a hyperplasia and proliferation of the epithelium of ducts is induced. The abnormal reaction of the mammary glands could not be restored towards normal by injections of testosterone into gonadectomized hypophysectomized rats treated with insulin and cortisone with or without thyroxine (Donovan & Jacobsohn, 1960*a*, *b*; Jacobsohn, 1960).

Observations on castrated male rabbits showed that testosterone does not elicit mammary growth, unless given in extremely high doses or together with oestrone. The effect, if any, of testosterone in physiological amounts was largely dependent on the dose of oestrone (Bengtsson & Norgren, 1960).

The failure of testosterone in hypophysectomized rats to induce mammary growth similar to that occurring in male rats with intact pituitary glands might then be due to a reduction of the blood level of oestrogenic hormones, the formation of which is not restored by the treatment with thyroxine, insulin and cortisone. This possibility was studied in the present work on hypophysectomized male rats injected with pregnant mare serum gonadotrophin (PMS) and on spayed hypophysectomized female rats injected with testosterone.

The schedule of injections and the doses of PMS, testosterone, thyroxine, insulin and cortisone were the same as in previous work (Donovan & Jacobsohn, 1960*a*, *b*). In the present experiments daily injections of

0·5 μg oestradiol benzoate for 2 days, followed by 0·05 or 0·1 μg oestrone, were added to the treatment.

The results obtained from experiments with PMS, thyroxine, insulin and cortisone in various combinations were as follows: (1) PMS stimulated the growth and hormonal activity of the testes and induced the same abnormal reactions of the mammary glands as testosterone under comparable conditions. (2) The addition of oestrogens modified the reaction of the mammary glands to PMS in the group treated with thyroxine, insulin and cortisone. Alveolar lobules, but no end buds were present in the glands of all rats of this group as well as in those of another group receiving the same treatment but without thyroxine.

Testosterone injected together with oestrogens into gonadectomized hypophysectomized rats treated with insulin and cortisone also induced a development of alveolar lobules. The addition of oestrogens did not, however, modify the reaction of the glands to testosterone when insulin was omitted.

The extent of alveolar development was less than in incompletely- or non-hypophysectomized rats. The present observations indicate, nevertheless, that the growth reaction of the rat's mammary gland to androgens is, as in rabbits, dependent on concomitant actions of oestrogens. The results obtained from the different groups support the view that the general metabolism is important for the growth of the mammary glands.

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The physiological basis of habituation. A preliminary showing of the effects of cerebral lesions upon habituation. By E. M. GLASER and J. P. GRIFFIN. *Department of Physiology, London Hospital Medical School, London, E.* 1

Evidence on selective inhibition of vagally induced gastric secretion caused by hyperglycaemia. By G. DOTEVALL and A. MUREN. *Medical Clinical I, University Hospital, and Department of Physiology, University of Gothenburg, Sweden*

It is well known that *hypoglycaemia* provokes gastric secretion by increasing vagal activity; and the insulin test has accordingly been widely used as a method to judge whether the vagal innervation to the stomach is

intact or not. As regards the effect of *hyperglycaemia* the information is limited, but inhibitory effects of intravenously administered glucose have been reported (Babkin, 1950). According to studies on clinical material, the incidence of gastric over-secretion and peptic ulcer is very low in diabetes mellitus (Joslin, Root, White & Marble, 1959; Dotevall, 1959). Recent studies have even shown that the basal secretion of hydrochloric acid is significantly lower in diabetics than in normal individuals (Dotevall, 1960).

The effects of intravenous infusion of glucose have been studied in dogs with Pavlov or Heidenhain pouches in which gastric secretion has been induced by different methods. So far, the following ways of stimulation have been used: insulin, sham-feeding or teasing, feeding and histamine. Gastric juice volume and acidity were estimated at 15 min intervals during periods of up to 8 hr. After stimulation, 30% glucose solution was infused intravenously, causing a blood sugar level of 300–400 mg/100 ml. The influence of glucose on secretory volume and acidity observed in these experiments was the following: (*a*) complete inhibition after insulin or during sham-feeding in Pavlov-pouch dogs; (*b*) partial or no inhibition after feeding in Pavlov-pouch dogs, depending on how soon after feeding glucose was given; (*c*) no inhibition after feeding in Heidenhain-pouch dogs; (*d*) no inhibition at all after histamine.

The present observations suggest that hyperglycaemia selectively inhibits or blocks the cephalic phase of gastric secretion. A consequence of this would be that intravenous infusion of glucose offers a simple method for quantitative distinction between the cephalic and the gastric phases of secretion.

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A simplified method of recording the effect of conditioning volleys on monosynaptic discharges. By BIRGITTA HOLMQVIST, A. LUNDBERG and P. VOORHOEVE. *Institute of Physiology, University of Lund, Sweden*

The ventilation of human lungs under standardized breathing conditions. By R. JERNÉRUS, S. LICHTNECKERT, C. LUNDGREN and G. LUNDIN. *Institute of Physiology, Division of Aviation Medicine, University of Lund, Sweden*

Histamine-induced bronchoconstriction in man. By A. Bouhuys, C. B. McKerrow and P. D. Oldham. *Institute of Physiology, University of Lund, Sweden*

Some physical and physiological principles in the design of breathing apparatus for frogmen. By M. Arborelius, Jr. and C. Lundgren. *Institute of Physiology, University of Lund, Sweden*

The role of the chemoreceptors in the cardiovascular responses to systemic hypoxia in the dog. By M. de Burgh Daly and Mary J. Scott. *Department of Physiology, St Bartholomew's Hospital Medical College, London, E.C.* 1

We have shown previously that in the dog the primary cardiovascular reflex effects of carotid body chemoreceptor stimulation are bradycardia, a decrease in cardiac output and an increase in total peripheral vascular resistance (Daly & Scott, 1958, 1959). These effects, however, are usually masked as a result of events arising secondarily from the concomitant reflex stimulation of respiration. The secondary effects are tachycardia, an increase in cardiac output and a reduction in total peripheral vascular resistance. In these experiments the isolated perfused carotid bodies were stimulated by hypoxic blood; the animals breathed room air, and the medullary centres therefore received oxygenated blood.

The aim of the present experiments was to find out what part, if any, the chemoreceptors play in the cardiovascular responses to systemic hypoxia.

The method used was similar in principle to that employed by Neil (1956). Dogs, anaesthetized with chloralose and urethane following premedication with morphine hydrochloride, were made hypoxic by substituting 7–12% O_2 in N_2 for room air. During hypoxia the chemoreceptor drive was abolished by changing the carotid body perfusate from hypoxic blood to oxygenated blood obtained from a donor animal (Daly & Scott, 1958). Cardiac output was measured by the dye-dilution method.

It was found that with the carotid bodies perfused from the same animal, systemic hypoxia usually caused an increase in respiratory minute volume, tachycardia, an increase in cardiac output and a reduction in total peripheral vascular resistance.

The carotid body perfusate was then changed from hypoxic recipient blood to oxygenated donor blood while the recipient animal continued to breathe the low-oxygen gas mixture. This caused a reduction in respiratory minute volume, a further increase in heart rate and in cardiac output; the

total peripheral vascular resistance decreased. When hypoxic blood perfusion of the carotid bodies was re-established these effects were reversed.

These results indicate that the cardiovascular effects of systemic hypoxia cannot be attributed to stimulation of chemoreceptors; indeed they actually antagonize the responses. These findings fall into line with those of Neil (1956) and of Daly & Scott (1959) who, using the cat and dog respectively, concluded that the carotid bodies do not contribute to the production of the tachycardia of systemic hypoxia.

The mechanisms responsible for the cardiovascular effects observed in systemic hypoxia remain obscure.

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Chemoreceptor impulse activity evoked by perfusion of the glomus at various pCO_2 and pH values. By N. Joels and E. Neil. *Department of Physiology, Middlesex Hospital Medical School, London, W.* 1

In anaesthetized cats the carotid body was vascularly isolated and perfused via the common carotid artery by Krebs–Henseleit solutions at constant temperature and hydrostatic pressure. All solutions were equilibrated with known partial pressures of CO_2 and O_2; their pH was measured by a glass electrode. By adjusting the bicarbonate concentration the pCO_2 could be varied without affecting the pH, or alternatively the pH could be changed, although the pCO_2 remained constant.

Impulse activity in pure chemoreceptor preparations was recorded as described by Heymans & Neil (1958).

Effects of changing pCO_2 in the isohydric perfusate. A rise of pCO_2 increased chemoreceptor impulse activity. The stimulant effect of raised pCO_2 is therefore not wholly dependent upon changes in extracellular pH. This effect of CO_2 occurred whether the pO_2 of the perfusate was high or low.

Effects of changing pH of the perfusate, pCO_2 remaining steady. Chemoreceptor impulse activity increased as the pH fell. Again this effect was obtained whether the pO_2 of the perfusate was high or low. The response, indeed, of the chemoreceptors to anoxia was surprisingly feeble if the pH of the perfusate was very alkaline, e.g. 7·75.

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The effect of capsaicine on pulmonary arterial baroreceptors.
By J. C. G. Coleridge and C. Kidd. *Department of Physiology, School of Medicine, Leeds 2*

Observations on blood flow in the myocardium of the rabbit.
By J. Grayson and D. Mendel. *Department of Physiology, University College, Ibadan, Nigeria*

The technique of internal calorimetry has been applied to the measurement of blood flow in the myocardium of the rabbit (Grayson & Mendel, 1960). In the present work observations have also been made on the dog and the baboon (*Papio anubis*). Recorders were inserted at open operation directly into the myocardium of the left ventricle. Coronary circulation was at no time interrupted or impeded and trauma to the heart and its nerve supply was minimal.

In the rabbit at systemic blood pressures of 90 mm Hg, the average δk value (difference between thermal conductivity of living and dead muscle) was 21×10^{-4} c.g.s. units; approximately $2\frac{1}{2}$ times greater than corresponding values for rabbit liver, suggesting a correspondingly higher blood flow per unit mass of tissue.

Mean systemic pressure was controlled by a compensator connected to a femoral artery (Grayson & Johnson, 1953). Blood pressure was lowered in stages from 90 mm Hg to zero. In the rabbit, where pulse rate did not vary until very low pressures were reached, linearity was found between pressure and flow (Grayson & Mendel, 1960). In both dog and monkey, where pulse rate rose slightly with falling pressures, a similar linearity was found, suggesting that in the dog and monkey, as in the rabbit, systemic blood pressure is a main determinant of myocardial blood. There was no evidence of autoregulation or of effective critical closing.

In the rabbit evidence has been adduced suggesting that sympathetic activity maintains a marked degree of myocardial resistance to blood flow (Grayson & Mendel, 1960). In the dog and monkey sympathetic blockade with Rogitine (CIBA) produced a marked rise in myocardial blood flow, although linearity between pressure and flow was not affected. Nor was the linearity affected by atropine or vagal section.

It must be concluded, then, that the diminution which occurred in flow with falling pressure was not due to nervous activity. It would also seem likely that in dog and monkey, as in rabbit, tonic sympathetic activity plays some part in maintaining myocardial resistance to blood flow. This may not be a simple vascular response, however, since in the rabbit there is good evidence that rate and force of contraction are also important

determinants of blood flow. The same probably holds true in dog and monkey, although the present evidence is incomplete.

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A mechanism for an ectopic pace-maker in anoxic rat heart. By J. F. LAMB. *Department of Veterinary Physiology, University of Edinburgh*

During experiments with DNP on rat auricles (Lamb, 1960) it was observed that two stimuli gave rise to repetitive activity in the tissue. Experiments were therefore carried out to specify the conditions under which this arose.

Anoxia or DNP shortens the action potentials of both auricles and ventricles and eventually leads to very short action potentials with low excitability. When the action potential is shortened to the intermediate durations of 20–30 msec, repetitive activity can be set up by the application of a second stimulus during repolarization. This second stimulus requires to be just above threshold and separated from the first by 20–30 msec. No activity can be set up when the action potential is shortened to less than 10–15 msec or is of normal duration. This repetitive activity is regular, at rates of 1200–2000/min, and lasts for a few seconds to several minutes.

As fast rhythms can also be set up in auricular tissue if the action potential is shortened by ACh (Hoffman & Suckling, 1953) or by reducing the external potassium concentration to zero (unpublished observations), it appears to be necessary for the production of these rhythms to shorten the action potential, but other factors may be involved in their production.

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The retention of wet aerosols in the human lung. By H. HERXHEIMER and E. STRESEMANN. *Asthma Poliklinik, Free University of Berlin, Rudolf–Virchow–Krankenhaus, Germany*

The retention of aerosols in the lung has previously been investigated mainly by nephelometry or thermo-precipitation. We have used an electrofilter (Cottrell filter) in which the aerosol particles are electrically charged by a high-voltage current and deposited on the cathode, a thin

aluminium foil (Wilner, 1951). The aerosol consisted of a 1% methylene-blue solution with 15% glycerine in water. The aerosolizers used were a commercial nebulizer (mean particle size of 1–1·5 μ) and the 'scrubber' (Dautrebande, 1951; mean particle size probably 0·2–0·5 μ). The deposit washed off the aluminium foil was determined by a photocolorimeter. The breathing of three normal subjects was registered on a recording spirometer with attached nebulizers (Herxheimer, 1949). Respiration rate and depth were either left to the choice of the subject or rigidly controlled by restricting the movements of the spirometer bell and by metronome. In some experiments with larger tidal air the breath was held for a period equalling the time required for half the respiratory cycle.

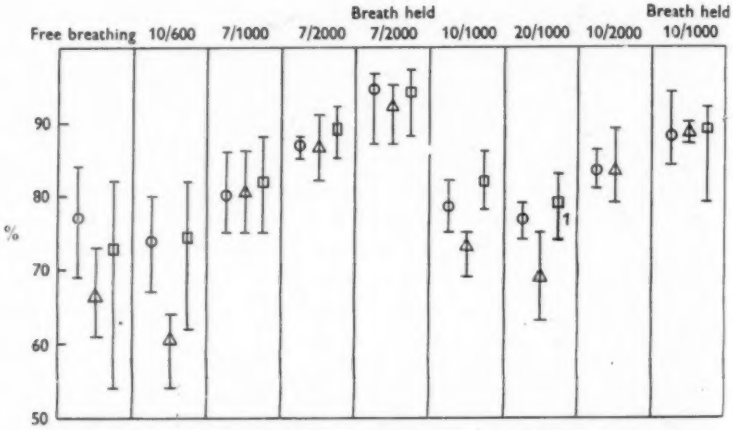

Fig. 1. Percentage retention (ordinate) in various types of respiration (abscissa). Respiratory rate varying from 7 to 20/min, tidal air from 600 ml. to 2 l. (1 = this subject has been breathing only 15/1000.)

According to the table the lung retention of particles in one subject is higher than 60%, and higher than 70% in the other subjects for all types of respiration. It is lowest with a small tidal air and increases with the tidal air to a retention between 80 and 90%. When breath is held in inspiration, retention often exceeds 90%. There was no spectacular difference between the aerosols of different particle sizes.

It is open to question in which part of the respiratory tract the retention occurred, but according to Findeisen (1935) it is likely that these small particles were retained in the lower part of it. The method may be of value for the bronchial testing of bronchoconstrictor and bronchodilator aerosols as well as of allergenic aerosols in normal subjects and in allergic patients.

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The failure of inhibitors of aerobic metabolism to prevent the anaphylactic release of histamine from guinea-pig lung. By K. F. AUSTEN* and W. E. BROCKLEHURST. *National Institute for Medical Research, Mill Hill, London, N.W. 7*

The view that antigen-induced histamine release requires aerobic metabolism is based mainly on studies of sensitized guinea-pig lung using cyanide (CN) or a nitrogen atmosphere (Mongar & Schild, 1957). The high concentration of CN used, 2–10 mM, is sufficient to inhibit a large number of enzymes other than cytochrome oxidase, which, in yeast and heart-muscle systems, is totally inhibited by 0·1 mM or less. We have carried out studies with carbon monoxide (CO) which inhibits cytochrome oxidase but affects few other enzymes (Dixon & Webb, 1958).

Incubation of perfused, chopped guinea-pig lung in a CO atmosphere in the dark for 15 or 30 min before and also during the anaphylactic reaction did not significantly diminish the release of histamine. Similarly, incubation in a nitrogen atmosphere for 15–60 min failed to diminish histamine release. 2-Heptyl-4-hydroxyquinoline-*N*-oxide 0·1 mM, was not inhibitory, even though one-fiftieth of this concentration produced significant inhibition of the cytochrome system in homogenized pig heart muscle (Lightbown & Jackson, 1955).

In further experiments we used the isolated perfused whole lung. Wet CO was bubbled into the perfusion fluid and was simultaneously administered at constant pressure into the trachea from which it passed through the lung and emerged via 15–20 surface scratches. One lobe of the lung was removed as a control before carbon monoxide treatment. The amount of acetylcholine (ACh) required to cause moderate bronchoconstriction rose during CO administration, suggesting some depletion of the energy stores; antigen was not administered until the effective dose of ACh had risen six- to tenfold. After 15 or 60 min of CO administration, the antigen injection produced maximal bronchoconstriction and released a greater proportion of the tissue histamine than was released when the untreated control lobe was chopped and incubated with antigen.

When CN 1 mM was added to chopped lung in stoppered tubes, and the antigen added immediately afterwards, about 70% inhibition of histamine

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release occurred. Incubation for 15 min before adding the antigen did not increase the inhibition, although the CN concentration remained practically unchanged.

The failure of CO to inhibit anaphylactic release of histamine indicates that cytochrome oxidase is not essential to the reaction. Furthermore, the observation that CN inhibition does not require pre-incubation of the tissue with CN suggests that this inhibition does not result from depletion of energy stores but rather from inactivation of enzymes other than cytochrome oxidase. Other workers have found that a nitrogen atmosphere did not inhibit antigen-induced rat mast-cell degranulation (Hogberg & Uvnas, 1960), and 5 mM CN was needed to inhibit this reaction (Mota, 1960). Thus, in the rat as in the guinea-pig the effect of CN might well be unrelated to inhibition of aerobic metabolism.

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Uptake of radioactive carbon dioxide by the lung. By C. T. Dollery and J. B. West. *Department of Medicine, Post-graduate Medical School, Hammersmith Hospital, London, W.* 12

Carbon dioxide has been labelled with oxygen-15, a cyclotron-produced isotope with a half-life of 2 min. After a single breath of the gas, the activity within a region of the lung can be measured by counters over the chest. Because the gas is highly diffusible, the clearance rate during breath-holding has been used as a measure of pulmonary blood flow, which has been shown to increase steadily from the apex to the base of the lung. This inequality of blood flow apparently accounts for most of the ventilation: perfusion ratio inequality in normal subjects (West & Dollery, 1960).

Oxygen-15-labelled carbon dioxide exhibits different physiological behaviour from carbon dioxide labelled with carbon-11, another short-lived cyclotron-produced isotope. For example, the clearance rates of the oxygen-labelled gas are about twice as fast as those of carbon-labelled carbon dioxide. Measurements on alveolar gas expired immediately after inspiration of radioactive carbon dioxide show that the oxygen-labelled gas disappears from the alveoli much faster than its carbon-labelled counterpart. Furthermore, if venous blood is drawn shortly after a breath of oxygen-labelled carbon dioxide, the activity cannot be removed by

adding acid and shaking under vacuum, whereas the expected loss of activity occurs for the carbon-labelled gas.

These apparent discrepancies can be explained by exchange between the oxygen atoms of water and bicarbonate after the gas has been taken up by the blood. *In vitro* measurements show that the uptake of oxygen-labelled carbon dioxide into blood can be reduced by adding carbonic anhydrase inhibitor. The results affect the interpretation of clearance rates of other radioactive gases in the lung.

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The measurement of the pulmonary capillary blood volume and membrane diffusion component on exercise. By D. V. BATES, R. V. CHRISTIE and C. J. VARVIS. *Department of Medicine, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada*

By measuring the lung-diffusing capacity for CO ($D_{L_{CO}}$) at differing alveolar oxygen tension, it is possible to compute the pulmonary capillary blood volume (Vc) and membrane diffusion components (Dm) in subjects with normal intrapulmonary gas distribution, making use of the kinetic data of Roughton & Forster (1957). To date, these components have only been measured by the single-breath modified Krogh technique (Lewis, Lin, Noe & Komisaruk, 1958; MacNeill, Rankin & Forster, 1958). Very few measurements made during physical exercise have been reported.

These components have been measured by a steady-state diffusing capacity technique during continuous exercise in fourteen normal subjects. The main conclusions which can be drawn from the data are as follows.

(1) Repeated observations in four normal subjects show that the pulmonary capillary blood volume (Vc) may be measured with a standard deviation of ± 12 ml. under these conditions. At 3 miles (4·8 km)/hr on the flat, with a mean oxygen uptake of 1·2 l./min, the pulmonary capillary blood volume averaged 140 ml.

(2) The relationship between the reciprocal of the measured total diffusing capacity (D_L) and Roughton & Forster's constant θ has been shown to be linear between oxygen tensions of 80 and 600 mm Hg.

(3) The membrane diffusion component averaged 51 ml. CO/min/mm Hg under similar conditions. The standard deviation of repeated measurements of this component in four normal subjects varied from $\pm 1{\cdot}5$ to $\pm 11{\cdot}8$ ml. CO/min/mm Hg.

(4) The value obtained for the pulmonary capillary blood volume is not affected by the method adopted to compute the mean alveolar CO tension, provided that the same technique is used in two determinations at the two chosen oxygen tensions. Since this alveolar CO computation is the most questionable part of the steady-state determination of diffusing capacity, it is felt that less reliance can be placed on measurements of membrane component by this technique, since these are sensitive to the method of computation of mean alveolar CO tension. It has also been shown that the calculated back-pressure effect of CO must not exceed 15% of the alveolar CO tension, if large random variations in calculated pulmonary capillary blood volume are to be avoided. For this reason higher concentrations of inspired carbon monoxide must be used for the determination during which 100% oxygen is breathed, and this precaution becomes of major importance in the case of heavy smokers who may be found to have a relatively high concentration of CO in the blood before the experiment is begun.

It is concluded that provided these precautions are kept in mind, the measurement of these components of diffusing capacity during steady-state exercise promises to be a repeatable and reliable technique for the study of factors limiting diffusion processes in the normal lung.

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Volume changes in active muscle. By B. C. ABBOTT and R. J. BASKIN. *Department of Zoology, University of California, Los Angeles, Los Angeles* 24, *California, U.S.A.*

The volume changes have been recorded during a single twitch of an isolated frog sartorius muscle. In order to measure the small, rapid volume changes, special instruments were developed to record the movement of a liquid surface as a change in capacitance.

An early volume increase was followed by a slower decrease in volume. The magnitude of these changes is a function of the contraction parameters (isometric or isotonic), of the length of the muscle, and also of the temperature. At 20° C with the muscle held isometrically at reference length, the volume increase starts about 2 msec after the stimulus and reaches a maximum at about 6 msec. The volume decrease then appears and reaches its minimum at approximately 9 msec after the stimulus. The magnitude of the volume increase was about $2{\cdot}5 \times 10^{-6}$ ml./g and the decrease amounted to about $5{\cdot}0 \times 10^{-6}$ ml./g.

If the muscle was held about 4–6 mm above reference length, the volume increase did not appear, and only the decrease was noted. If the muscle was held isometrically below reference length, the volume increase was greater and the decrease was less than at the greater length. The dependency of the volume changes upon the length of the muscle explains discrepancies in these measurements as made by Ernst (1925), Meyerhof (1934), and Fischer (1941), where no particular attention was given to muscle length.

The results at low temperatures are very striking. At 2° C the early volume increase started within 5 msec after the stimulus and reached a peak at 10 msec. The volume decrease reached its minimum at about 30 msec after the stimulus, or about 50 msec before the tension peak of the twitch. It had been noted by previous investigators (Meyerhof, 1934) that even after a single twitch (at 15° C) the volume did not completely return after the decrease. The present experiments showed that the magnitude of this 'permanent' volume decrease is a function of temperature. At about 2° C the volume did not recover at all from the decrease within the duration of the twitch. This means that the 'permanent' volume decrease retains (at about 2° C) a magnitude of $5{\cdot}0 \times 10^{-6}$ ml./g and cannot be a function of active pressure developed inside the muscle.

The time relationships of the changes suggest that the early increase in volume corresponds to the latency relaxation and so to the initial coupling events; the decrease corresponds to active state rather than to tension development; and the long-lasting decrease to the break-down of creatine phosphate.

The constancy of the diastolic length-tension relationship in strips of ventricle. By F. T. Moore and E. Ogden. *Department of Physiology, The Ohio State University, Columbus, Ohio, U.S.A.*

The change of tension associated with changes of length was measured in strips of ventricle from rats and frogs. The range was 50% additional length for frogs, 100% for rats. Within this range, which by comparison with other work does not constitute an excessive stretch, the force–stretch diagram for each heart was closely reproducible in experiments where the electrical excitation was arranged to allow a long diastole (the rat ventricle was stimulated once each second and 5 min elapsed between any two series of stretches. Stimuli were applied to the frog at 1 min intervals).

Changing the temperature, addition of Nembutal (pentobarbitone sodium; Abbott Laboratories), addition of adrenaline and of the combination of Nembutal and adrenaline were all applied sufficiently to produce unquestionable systolic effects in the expected direction.

Regardless of the effect in systole the diastolic behaviour of the hearts was unchanged. The relationship is discussed between the implications of this work and that of other work from this laboratory (e.g. Buckley, Sidky & Ogden, 1956) which indicates that the heart which is about to eject a large charge of blood is more easily filled than otherwise.

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Bradykinin as a vasodilator in man. By R. H. FOX, R. GOLDSMITH D. J. KIDD and G. P. LEWIS. *Division of Human Physiology, and Division of Physiology and Pharmacology, National Institute for Medical Research, London, N.W. 3*

The pure polypeptide bradykinin, as isolated by Elliott, Lewis & Horton (1959), has a strong vasodilator action in man. The effect was obtained in seventeen experiments on twelve adult males. Total forearm or hand blood flows were measured by venous occlusion plethysmography. Surface calorimeters were used to detect changes in skin blood flow in the face or limbs. Intra-arterial injection of as little as 0·1 μg bradykinin sometimes produced an increase in hand or forearm blood flow; 1·0 μg always produced a large vasodilatation, and in one subject this dose increased forearm blood flow from 5 to 33 ml./100 ml. tissue/min.

In four experiments on the forearm the magnitude of the vasodilator responses to intra-arterial injections of bradykinin were compared with those to injections of histamine and acetylcholine. Weight for weight the vasodilatation produced by bradykinin was usually equivalent to or a little larger than that produced by histamine, and much larger than that of acetylcholine. In four experiments in which hand blood flow was measured the vasodilator potencies of arterial injections of bradykinin and histamine were again found to be approximately equal. Between 10 and 100 times greater doses of acetylcholine were required to produce vasodilatation, probably because acetylcholine is rapidly destroyed in the blood stream (Duff, Greenfield, Shepherd & Thompson, 1953).

Following the intravenous injection or infusion of bradykinin, the earliest symptom was usually a sensation of flushing in the face; with higher doses the sensation became pronounced and the flushing became visible. In some subjects a short-lived choking sensation, an awareness of cardiac action, mild sensations of cold or warmth over most of the body surface, pulsations of the lips and a metallic taste in the mouth were reported.

On intravenous infusion, bradykinin was found to be less active than

histamine in producing vasodilatation. For instance, in one experiment infusion of bradykinin 52·5 μg/min and histamine 9 μg/min produced approximately equal vasodilatations in both the hand and the forearm. Both substances also caused large increases in blood flow through the skin of the cheek and ear. The difference between intra-arterial and intravenous dose responses probably indicates a more rapid destruction of bradykinin than of histamine in the blood stream.

Bradykinin administered intra-arterially or intravenously to man is a potent vasodilator substance and in these experiments no other effects were observed.

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A technique to demonstrate the manner in which arteries are compressed when exposed to external pressures, in subjects with 'hardened' arteries. By I. F. S. Mackay. *Department of Physiology, University College of the West Indies, Jamaica*

The basis of the technique was to employ a method devised to measure 'arterial' flow. It consists of the drainage of the blood vessels by elevating the limb. This is immediately followed with the application of an occluding pressure tourniquet at 210 mm Hg. The limb is now placed in the dependently vertical position where the pressure in the arteries falls close to zero. On release of the tourniquet an initial flow rate 5 to 10 times that recorded by venous occlusion plethysmography is obtained. A light forearm and hand plethysmograph was used. As it is considered that the factors involved in the resistance to the initial flow may be more in the larger vessels than in the smaller resistance vessels, it has been called 'arterial' flow. This method has already been described (Mackay, 1955). A series of such 'arterial' blood flows were recorded where the occluding cuff was released to residual pressures from zero up to pressures which prevented any flow. The 'arterial' flow rates are used to indicate the degree of compression of the artery. These flow values, when plotted against the residual pressures, produced curves which were drawn in order to indicate the manner of the collapse of the artery to various external cuff pressures.

The shapes of these curves show that in normal subjects the artery is not compressed by cuff pressures up to approximately the diastolic pressure. With higher pressures, there is initially a rapid collapse which becomes more gradual as the cuff pressures are increased. The curve of collapse is concave facing upwards (Mackay, 1957).

The traditional method of measuring blood flow by venous occlusion plethysmography was also employed, with various occluding cuff pressures. A similar shape of curve is obtained, but the point where collapse commences is often at a higher pressure.

In subjects clinically designated as having 'hardened' arteries, the results from the above two methods were different. With the 'arterial' flow method the curve of collapse has a concavity facing downwards. At first, the fall of the curve is gradual and there appears to be a resistance to compression. Then, at higher pressures there is a more rapid decline of the curve.

In these pathological subjects the venous occlusion plethysmographic method gives a curve of similar shape but the point where the curve starts to fall is at a much higher pressure than that obtained by the 'arterial' method. Thus, in pathological subjects there is a larger pressure difference between the points obtained by the two methods at which the flow rates start to fall than in normal subjects. It is suggested that the 'arterial' method indicates the resistance to compression of 'hardened' arteries.

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The input impedance of the pulmonary vascular bed. By D. H. BERGEL, C. G. CARO and D. A. MCDONALD. *Department of Physiology, St Bartholomew's Hospital Medical College, London, E.C.* 1

The pressure at the origin of the pulmonary artery was measured while a sinusoidal flow was created by a pump. The ratio of this pressure to the rate of flow is the input impedance. Fluctuations in this impedance in a 'closed' system such as a vascular bed will be largely determined by the presence of reflexions. Their effect depends on the relation between the length of the system and the wave-length at any given frequency. The input impedance is at a minimum when the system is one quarter wave-length long and maximal when it is a half wave-length. Measurement on the input impedance over a range of frequencies will thus describe the normal relations between pressure and flow in the pulmonary trunk and demonstrate any effects that reflexions may produce (McDonald, 1960).

Isolated rabbit lungs (twenty-five animals) were perfused with isotonic saline or dextran from a high-pressure reservoir connected through a reducing valve. This regulated the steady flow which was measured by a meter in the high-pressure line and the valve excluded the impedance of the tubing and reservoir from the system. The resistance of the bed varied

from $1{\cdot}5$–$4{\cdot}0 \times 10^3$ dyn. sec/cm^5 up to very high values after the onset of oedema, or after embolization of the capillaries.

The range of frequencies studied was from 1 to 20 c/s. The input impedance in all cases was at a minimum around 4 c/s (3–5). The mean value at this minimum was $0{\cdot}73 \times 10^3$ dyn. sec/cm$^5 \pm 0{\cdot}11$ (S.E.). At lower frequencies the impedance rose sharply and was 4–10 times greater at 1·0 c/s. It also rose above 4 c/s and in most cases (16 expts.) reached a maximum at about 8 c/s (7–10). The ratio of this maximum to the minimum was $2{\cdot}67 \pm 0{\cdot}2$ (S.E.).

These findings show that reflexions were occurring and the minimum at 4 c/s is interpreted as indicating that the effective length of the bed was one quarter wave-length at this frequency. From the measured wave-velocity (Caro & McDonald, 1960) of 90 cm/sec the calculated average length is 5·5–6·0 cm. A maximum at 8 c/s then corresponds to a half wave-length. Changes in resistance bore little relation to changes of impedance, which suggests that the main reflecting sites are proximal to the 'resistance' vessels even though some pulsation may be transmitted to the veins.

It is remarkable that a clear-cut minimum was found despite the greatly varying lengths of the arteries to different lobes of the lungs. It is also interesting that the impedance was smallest at a frequency close to the normal heart rate, so that the impedance pattern may play a part in determining the optimal pulse frequency.

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Formation of plasma kinin in human plasma by ε-amino caproic acid and certain related compounds. By DESIRÉE ARMSTRONG and J. W. STEWART. *Middlesex Hospital Medical School, London, W.* 1

ε-Amino caproic acid inhibits both the activation of plasminogen and the proteolytic actions of plasmin and trypsin (Alkjaersig, Fletcher & Sherry, 1959). Lewis (1958) suggested that plasmin is responsible for the plasma kinin formation observed when human plasma comes into contact with glass (Armstrong, Jepson, Keele & Stewart, 1957). ε-Amino caproic acid was tested for its inhibitory action on this kinin formation and none was demonstrated. In fact, ε-amino caproic acid (final concentration 0·01–0·12 M) induced kinin formation (isolated rat uterus test) when added to human pre-active plasma in polythene. The acid itself was not oxytocic, but the mixture with plasma developed detectable oxytocic activity after a few minutes incubation at 20° C—maximal kinin activity being recorded

after ½–1 hr. Higher concentrations of amino acid induced more rapid kinin formation.

By the blister-base technique, mixtures of amino acid and pre-active plasma were shown to cause pain after suitable incubation.

Oxytocic and pain-producing agent was extractable with boiling ethyl alcohol from suitably incubated, but not from fresh, mixtures of acid and plasma, and incubation, at 20° C, of active extracts with a bacterial (*Escherichia coli*) amino-peptidase preparation resulted in rapid destruction of the oxytocic activity.

Other amino acids gave similar results. α-Amino acids were somewhat less active than the corresponding ω-amino acids, and diamino acids, such as lysine, were slightly more active. Of greater importance, however, was the carbon chain length of the acid, the longer chain acids (e.g. ω-amino caprylic acid) having the greater activity. Both the NH_2 and COOH groups are essential for this kinin formation, since the aliphatic mono- and diamines tested induced little activity and the fatty acids themselves none.

Kinin formation by ε-amino caproic acid was greatly reduced in plasma which had been previously exposed to glass (this reduces 'component B', Margolis, 1958), and in the plasma of parturient women which is substrate-deficient (Armstrong, Keele & Stewart, 1960). It was inhibited by the presence in the plasma of the soya bean trypsin inhibitor, sodium fluoride or nucleic acid (thymus gland preparation), which also depressed the formation of kinin on exposure of human plasma to glass. Heparin promoted both. Kinin production by 'Thrombolysin' (Merck) was unaffected by heparin, fluoride or nucleic acid.

Plasma kinin formation by ε-amino caproic acid appears to be similar to that by glass exposure and both these mechanisms of kinin formation are independent of the plasminogen-plasmin system.

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Intrinsic plasma kinin forming factors in human plasma. By V. Eisen and C. A. Keele. *Middlesex Hospital Medical School, London, W.* 1

Plasma kinin formation has been studied in human plasma in which plasmin inhibitors have been inactivated by acidification to pH 2 for 15 min (Alkjaersig, Fletcher & Sherry, 1959; Rocha e Silva, 1960); by

treatment with acetone (Lassen, 1958) or chloroform (Tagnon, 1942); and by isolation of euglobulins (Alkjaersig *et al.* 1959). Plasma kinin was estimated by its stimulating effect on the isolated rat uterus and guinea-pig ileum and sometimes by the production of pain in an exposed blister base. In all these preparations 'spontaneous' kinin formation occurred, which was not accompanied by fibrinolytic activity (measured by lysis of human and bovine fibrin), or by proteolytic activity (measured by casein digestion). Conversely, the development of kinin-forming activity in a given preparation was slightly or not at all enhanced when its fibrinolytic and proteolytic potential was activated by streptokinase. In whole plasma, clot lysis and kinin formation could also proceed independently of each other.

This kinin formation was, however, largely dependent on the presence of the factors necessary for kinin release by glass. As postulated by Margolis (1958, 1960), these included Hageman factor (HF), and 'components A and B'. In the absence of any one of these factors plasma kinin was formed much more slowly than in the presence of all three factors.

Our work suggests that in addition to activation by adsorption on to suitable surfaces, HF can be activated in solution by the procedures mentioned above. It is, however, to be noted that acidification and precipitation by acetone impair the capacity of HF to be adsorbed on to glass.

A preparation of human plasmin called 'Thrombolysin' (Merck) contains a factor which in its kinin-forming properties resembles the soluble active form of HF. The rapid formation of kinin induced in intact human plasma by 'Thrombolysin' in concentrations of 0·05–1·0 mg/ml. seems to be due to this HF-like constituent and not, as previously stated (Eisen & Keele, 1960), to plasmin itself.

We have thus shown that procedures which are used to remove inhibitors of the fibrinolytic system and of serum kallikrein lead to activation of the factors which participate in the reactions of human plasma to foreign surfaces. In spite of certain common features, HF and A can be distinguished from plasmin since they do not digest fibrin and casein. They differ from serum kallikrein by their resistance to acid (Werle & Maier, 1952). 'Component A' differs from HF by its destruction by heating at 56° C for 1–3 hr. When fully activated this intrinsic HF, A, B system forms plasma kinin much more rapidly than does the fully activated plasminogen-plasmin system present in normal human plasma (cf. Lewis, 1958).

One of us (V. E.) is working on a grant from the Empire Rheumatism Council. We are grateful to Dr J. J. F. Merry of Merck Sharp and Dohme Ltd. for helping us obtain samples of 'Thrombolysin' and to Dr J. Margolis for sending us a sample of plasma deficient in Hageman factor.

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Interactions of a bacterial pyrogen with rabbit leucocytes and plasma. By K. E. COOPER, W. I. CRANSTON and J. H. FESSLER. *Medical Research Council Group for Research on Body Temperature Regulation, Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford*

The interaction between a bacterial pyrogen and leucocytes may be of importance in the production of fever (Cranston, 1960), and this process has been investigated *in vitro*. Leucocytes, obtained from rabbit peritoneal exudates (Cooper, Cranston & Fessler, 1960), were incubated at 37° C with a heat-stable, pyrogenic lipopolysaccharide, 'Pyrexal' (Wander) obtained from *S. abortus equi* (Westphal & Luderitz, 1954); the pyrogenic activity of the products was measured directly and after heating to 90° C for 30 min (Petersdorf & Bennett, 1957). If 0·1 μg or less of 'Pyrexal' was incubated with 2×10^7 leucocytes or more the pyrogenicity of the product was entirely abolished by heating (90° C for 30 min). When the proportion of 'Pyrexal' was raised an increasing amount of heat stable pyrogen remained. An incubation time of 1 hr was required, and the interaction occurred as well in 0·9 % NaCl as in a Krebs–Ringer solution, pH 7·3.

The interaction also occurred between 'Pyrexal' and ultrasonically disintegrated cells, or cells which had been stored in saline at 4° C for 1–3 weeks. Therefore the process does not require live, intact cells. Prolonged incubation of 'Pyrexal' with aged cells progressively reduced pyrogenicity of the mixture. Previous incubation of the cells with saline did not alter the interaction on subsequent incubation with 'Pyrexal'.

Rabbit leucocytes, obtained from peritoneal exudates, liberate pyrogen when incubated alone in saline at 37° C, and this process is inhibited by treating the donor animals with nitrofurazone (Cooper *et al.* 1960). In contrast, nitrofurazone treatment of the donors does not affect the subsequent interaction of the cells with 'Pyrexal'. Similarly, cells aged *in vitro* do not form any pyrogen when incubated alone, but interact with 'Pyrexal'.

The following properties of the heat labile fever principle resulting from incubation of 'Pyrexal' with fresh rabbit leucocytes were found:

(1) Optimum stability at pH 6–7·4.

(2) Can be precipitated with $(NH_4)_2SO_4$ and the activity subsequently recovered.

(3) Not inactivated by prolonged exposure to 5 M urea at 4° C.

(4) One pyrogenic dose contains less than 25 μg nitrogen.

When 'Pyrexal' is incubated with rabbit plasma an interaction also occurs resulting in a fever principle which is heat labile and causes fever in the 'Pyrexal' tolerant rabbit. 2·5 ml. plasma are required to react completely with 0·1 μg pyrexal, and the fever principle is completely precipitated by half saturation with $(NH_4)_2SO_4$.

Pyrogenic materials of similar biological properties were produced by two systems, one requiring the addition of bacterial pyrogen, and the other not. The evidence presented shows that there are other differences between these systems, and it therefore becomes questionable whether the similar biological properties are sufficient evidence for a conclusion of identity of the pyrogenic products.

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Tandem muscle spindles in the frog and cat. By D. Barker, M. Cope and M. C. Ip. *Department of Zoology, University of Hong Kong*

A tandem spindle in cat rectus femoris and flexor digitorum longus muscles typically consists of a large capsule with ten to twelve muscle fibres bearing one primary and two to three secondary endings, and one or more small capsules with three or four muscle fibres bearing a primary ending only. This is in contrast to the condition where a few single spindles may be conjoined side by side (compound type), or lie end to end with interdigitating poles. The most common tandem is the double kind in which two capsules are spaced along the length of the intrafusal bundle; of 64 tandem spindles traced in transverse section, 88% were double, 9% triple, and 3% quintuple. Only one or two muscle fibres run through the intercapsular regions to partake of successive encapsulation, and these are always of large diameter; the rest taper off and rarely interdigitate. A study of serial transverse sections of a pair of kitten rectus femoris muscles, and of twelve blocks sampled from the recti of six adult cats (lateral and medial

halves of the muscle each sampled six times, three times from each limb, four cats being used for sampling in the case of each half), indicates that 16–20% of the spindles in this muscle are of the tandem type. Length measurements of 32 tandem and 144 single spindles from cat's rectus femoris were 8·43–22·30 mm, mean 13·0 ± 3·24 mm, and 2·40–13·68 mm, mean 7·02 ± 2·45 mm, respectively.

Single spindles and double tandem spindles have been observed in the frog's extensor digitorum longus IV muscle, and a special study made of the 'spindle systems' of Gray (1957). Reconstructions made from serial transverse sections show that these measure 17·13–26·52 mm in 120–200 g frogs and consist of three to five intrafusal bundles lying closely side by side, each one usually extending throughout the length of the muscle and undergoing several encapsulations. In such a system each bundle may become separately encapsulated at the same level, or the bundles may unite temporarily in a common capsule; alternatively, at a level where some of the bundles have capsules, others in the system pass by without encapsulation. A bundle may be encapsulated from once to four times in its course through the system to which it belongs. In the systems studied there were from five to thirteen capsules so arranged as to constitute from three to four areas of sensory innervation. The morphology of these systems suggests that they could appropriately be termed compound tandem spindles.

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The effects of certain anaesthetics on the activity of gamma motoneurones serving the hind limb of the rat. By B. L. ANDREW. *Department of Physiology, Queen's College, Dundee*

Several factors influence the tonic activity and the reflex excitability of the lumbar gamma motoneurones in the intact, lightly anaesthetized rat. Three of these factors have been considered in the present experiments, namely, the anaesthetic agent used, the amount administered, and the degree of sensory stimulation.

In the experiments to be described, a basal anaesthetic (urethene, thiopentone sodium or pentobarbitone sodium) was injected intraperitoneally and filaments were dissected from motor nerves to hind-limb muscles. When a preparation containing one or two gamma efferents had been obtained, and the tonic resting activity, if any, and responsiveness to sensory stimulation had been assessed, further quantities of an

anaesthetic were given, either by injection or by inhalation. The tonic activity and reflex responsiveness were then re-examined.

The anaesthetics which have been tried have been divided into two categories. In the first category, which included trichlorethylene, ethyl chloride, chloroform and ether, the tonic activity was increased by the anaesthetic but at the same time the reflex responsiveness was decreased. The time course of the stimulant effect on the tonic activity showed some variation. The response to trichlorethylene was usually in two phases; an initial phase during the first minute of inhalation was probably due to the arousal effect of the smell and irritant properties of the vapour on the upper respiratory passages. This phase was transient and during the next 2 or 3 min the tonic gamma activity returned to the resting level. The second phase then began and consisted of an increasing excitation lasting several minutes and terminating abruptly with a few short volleys of impulses at high frequency, followed by quiescence. The reflex responsiveness of the gamma fibres to sensory stimulation had been abolished before the end of the second phase. The response to ethyl chloride was much more rapid, a single phase of excitation followed by abrupt extinction has been observed during the first minute of inhalation. These effects with inhaled anaesthetics are similar to those which have been described to occur in the decerebrate rabbit by Diete-Spiff & Pascoe (1959).

In the second category, which included urethane, thiopentone sodium and pentobarbitone sodium, there was found a depression of tonic activity and reflex sensitivity, but in the case of urethane large doses could be given before the spinal reflexes were abolished.

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A note on the classification of muscle spindle endings into types A1 and A2. By R. J. HARVEY* and P. B. C. MATTHEWS.
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Matthews (1933) classified muscle spindle endings into two types depending upon whether their discharge accelerated (A2) or slowed (A1) during supramaximal stimulation of the muscle nerve. He attributed this difference in response to the differential effects of contraction of intrafusal muscle fibres upon different endings. More recently the acceleration of the A2 discharge has been ascribed solely to the contraction of extrafusal muscle fibres (Kuffler, Hunt & Quilliam, 1951; Hunt & Kuffler, 1951; Hunt, 1954), though such a suggestion does not explain Matthews's results.

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In an attempt to clarify the position we have studied under isometric conditions the effects of tetanic stimulation of the muscle nerve on the discharge, recorded from a dorsal root filament, of de-efferented muscle spindles of the cat's soleus muscle.

Of fifty-seven endings studied the discharge of fifty-six accelerated when the nerve was stimulated at 70/sec with stimuli about ten times threshold for contraction and when the initial tension was appreciable (up to 500 g). This A2 type of discharge was shown by both primary and secondary endings, for the conduction velocities of the afferent fibres studied ranged from 30 to 116 m/sec(thirty-six primary, twenty-one secondary endings as classified by Hunt, 1954). The threshold stimulus required to produce acceleration varied, but was above that required to produce a maximal contraction and also above the threshold of the most excitable γ fibres (determined by later recording their compound action potential from a ventral root). Acceleration was not so marked when either the frequency of stimulation or the initial tension was reduced, and though it was usually still found with strong stimuli at 30/sec and a low initial tension (about 50 g), in some cases (usually secondary endings) it was not then demonstrable. In contrast to their behaviour in the rabbit (Diete-Spiff, 1960) some secondary endings could be made to fire one or two impulses on the rising phase of a twitch, if the stimulus was considerably supramaximal for the contraction and the initial tension was high.

The discharge of a sixth of the endings (eight primary, two secondary) accelerated slightly also on low-frequency stimulation (10–30/sec) with shocks too weak to excite γ fibres. On stimulation at 70/sec such weak acceleration was masked by direct stimulation of the afferent fibre. Matthews's A2 group may have included some endings so excited, but probably also contained some endings excited by stimulation of γ motor fibres, which for various reasons may have been stimulated in his experiments by relatively weak stimuli. It is concluded that under appropriate conditions all muscle spindle endings may be excited by the γ motor fibres to show the A2 type of response. The significance of the division into A1 and A2 endings which may be made with a restricted range of stimulating conditions is unknown.

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The effects of stretch on the tension and rate of discharge of crayfish stretch receptors. By K. KRNJEVIĆ and N. VAN GELDER*.
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Abdominal stretch receptors, together with the appropriate nerve, were dissected out in freshwater crayfish (*Astacus fluviatilis*). The receptors, which are modified muscle bundles (Florey & Florey, 1955), were clamped at one end with a fine forceps and tied at the other with wire to a glass hook attached to an RCA 5734 valve. The transducer and the forceps were mounted on a common threaded shaft and could be separated, stretching the receptor symmetrically. The afferent discharge was recorded from the nerve with 50 μ platinum electrodes.

The changes in tension observed during stretch agree with previous observations on isolated muscle fibres (Buchthal, 1942). When the receptor bundle was extended in steps of, for example, 100 μ, the tension initially developed only very slowly but then at a progressively faster rate. There was also clear evidence of hysteresis during relaxation. Unless stretching was very slow, a change in length was associated with an overshoot of tension which decayed to a plateau with a half-time of the order of a few seconds; the amount of overshoot bore an approximately constant relation to the final increment of tension. During relaxation a similar, but somewhat less pronounced undershoot was seen.

As a rule, when the bundle was stretched, there was a linear relation between the steady rate of firing of slowly-adapting receptors and the tension developed. During the dynamic phase (cf. Eyzaguirre & Kuffler, 1955; Florey, 1956) the rate of firing (F) was also linearly related to the peak tension (T), but with a steeper slope of $\Delta F/\Delta T$. There was no clear-cut distinction between the properties of slowly- and fast-adapting receptors, although the latter in general began to fire at a greater tension, and after each increment of stretch the tension was less well maintained. The maximal rate of discharge of fast-adapting receptors usually also had a linear relation to the peak tension.

It is suggested that the visco-elastic properties of the receptor bundle may be wholly responsible for the pattern of afferent discharge observed during moderate stretch and relaxation.

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Presynaptic inhibition in the spinal cord. By J. C. Eccles, Rosamond M. Eccles and F. Magni. *Department of Physiology, Australian National University, Canberra, Australia*

Frank & Fuortes (1957) and Frank (1959) have reported that a conditioning afferent volley in the nerve to biceps-semitendinosus produces a prolonged depression of the EPSP monosynaptically generated in a gastrocnemius motoneurone by the appropriate testing volley. In a further investigation of this phenomenon it has been shown that it is produced both by Ia and Ib afferent volleys, but not by Groups II or III. The depression is first seen with a testing interval of a few milliseconds, reaches a maximum in 10–20 msec, and then slowly decays. The depression of the EPSP is not associated with a change in its time course or in the resting potential of the membrane. On repetitive stimulation of the conditioning nerve, the EPSP depression becomes maximal after a few impulses and reaches a steady state which is maintained even during 60 sec tetanization. With increases in frequency up to 100/sec there is a commensurate increase in the depression, but there is little further increase as the frequency rises above 200/sec. Following a tetanus there is post-tetanic potentiation of the depression for about 60 sec. The large depression produced by repetitive conditioning volleys has aided investigation of the distribution of EPSP depression. It is produced mainly by volleys from flexors, and to a lesser extent from extensors, but the EPSPs of both flexor and extensor motoneurones exhibit the depression.

The depolarization of the presynaptic terminals of Ia fibres demonstrated in a recent Communication provides a satisfactory explanation of every aspect of these investigations on EPSP depression. There would be a diminution of the presynaptic spike potential and consequently a diminution in the liberation of excitatory synaptic transmitter and in the size of the EPSP, as has been shown by Hagiwara & Tasaki (1958) for the EPSP of the squid stellate ganglion. With the cool animal at low frequencies of stimulation depression would also arise on account of collision of the testing presynaptic volley with the centrifugal impulses of the dorsal root reflex. Thus in general the EPSP depression may properly be designated presynaptic in contrast to inhibitory action by post-synaptic changes that are produced by transmitter action from inhibitory synapses, and which may be designated post-synaptic inhibition.

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Latency of central inhibition. By T. Araki, J. C. Eccles and M. Ito.
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Afferent volleys in the sacral two or three dorsal roots inhibit the contralateral motoneurones with a central latency which is so brief that Lloyd & Wilson (1959) have been led to conclude that the central inhibitory path is monosynaptic and that the inhibitory post-synaptic potential (IPSP) of motoneurones is not the primary agent of this inhibition, but may be a secondary and later manifestation of the inhibition. These conclusions stand in contradiction to series of investigations in which the inhibition of monosynaptic reflex discharges was correlated with the potentials (IPSPs and EPSPs) recorded intracellularly from motoneurones and in which very strong evidence was produced that the Ia IPSP had a longer latency (by about 0·8 msec) than the Ia EPSP because there was an interneurone on the inhibitory pathway (cf. Eccles, 1957). In a re-investigation the latency of central inhibition was measured on the impulses discharged along motor axons in the ventral root, rather than on impulses recorded in the soma of motoneurones, a procedure that has never previously been adopted. When an inhibitory Ia volley precedes a monosynaptic reflex spike at an appropriate interval, only the later components of the reflex spike are delayed or suppressed by the inhibition. The onset of this inhibitory action on the spike thus gives a precise measure of the central latency of inhibitory action if it is corrected for the propagation time of the impulses from the central locus of inhibitory action to the recording site. With fast recording and superimposed traces of control and inhibited reflex spikes, the central inhibitory latency could be measured accurately. It was found always to be within 0·1 msec of the latency of the IPSP, both for the inhibitory action of quadriceps Ia volleys on monosynaptic reflex discharges from biceps-semitendinosus neurones and for the inhibitory action of contralateral S3 afferent volleys on monosynaptic reflexes in the ipsilateral S3 ventral root. Thus the answer is definitive. There is no experimental basis whatever for the postulate of Lloyd & Wilson (1959) that there is some earlier inhibitory process which has no electrical sign and which somewhat later produces the IPSP. The latency of the earliest central inhibitory process gives just the time that is required for the interneurone that is shown to be on the inhibitory pathway by several other lines of investigation (Eccles, Fatt & Landgren, 1956; R. M. Eccles & Lundberg, 1958).

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The synaptic delay at inhibitory synapses. By E. EIDE, A. LUNDBERG and P. VOORHOEVE. *Institute of Physiology, University of Lund, Sweden*

Ia fibres (with annulo-spiral endings on muscle spindles) have excitatory synaptic endings on motoneurones. The delay at these synapses is 0·3–0·5 msec (Lorente de Nó, 1935; Brock, Coombs & Eccles, 1952). Originally it was believed that reciprocal Ia inhibition was also exerted monosynaptically (Lloyd, 1946). However, intracellular recording from motoneurones revealed that the central latency of the Ia inhibitory post-synaptic potential (IPSP) is about 0·8 msec longer than for the Ia excitatory post-synaptic potential (EPSP) (Brock *et al.* 1952) and to account for this difference Eccles, Fatt & Landgren (1956) postulated that an interneurone is interpolated in the Ia inhibitory pathway and that the delay at inhibitory synapses is of the same order as at excitatory. They made the suggestion that all inhibitory pathways contain a last-stage short-axon inhibitory interneurone. In subsequent investigations on the C.N.S. no inhibitory pathway has been discovered with a briefer central latency than the Ia inhibitory pathway and the interpretation of central latencies in terms of number of relays has been based on the assumption of a brief delay at inhibitory synapses.

Indirect evidence supporting the interneurone hypothesis has been given (Eccles *et al.* 1956; Eccles & Lundberg, 1958), but it would be desirable to obtain more direct information about the delay at inhibitory synapses. If the hypothesis is correct it should be possible to stimulate these inhibitory interneurones electrically and thus evoke monosynaptic short-latency IPSP's in motoneurones. For this purpose the ventral part of the dorsal horn has been stimulated with electrodes inserted from the cord dorsum to a depth of 2·5–3 mm. Intracellular recording was made from motoneurones located ventral to the stimulating electrode. Large post-synaptic potentials were evoked in the motoneurones and with both IPSPs and EPSPs the latency was about 0·5 msec. The minimal latency observed was 0·46 msec for an IPSP and 0·50 for an EPSP. It is concluded that the delay at inhibitory synapses is of the same order as at excitatory and that the 'inhibitory interneurone hypothesis' is correct.

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The representation of the visual field on the optic tectum of the frog: evidence for the presence of an area centralis retinae.

By M. JACOBSON. *Department of Physiology, University of Edinburgh*

The representation of the visual field on the optic tectum of *Rana temporaria* was mapped by a method similar to that used by Gaze (1958*a*).

The frog was fixed with its right eye covered and its left eye centred on the axis of an A.I.M. projection perimeter. A spot of light subtending an angle of 10′ at the eye could be projected to any position in the frog's visual field. Action potentials evoked in response to the spot of light were recorded from the surface of the tectum with a steel micro-electrode (tip diameter about 5 μ) and amplified in the usual manner.

At each electrode position on the tectum there was a point in the visual field at which the stimulus evoked a maximal response. The determination of the position of maximal response was repeated with the electrode at successive positions spaced at 200 μ intervals on the tectum. Responses were obtained from 40 to 60 positions on the contralateral tectum and 10–20 positions on the ipsilateral tectum.

The results confirm the findings of Gaze (1958*a*, *b*) that there is a point-to-point projection of the visual field on to the optic tectum. There is a bilateral representation of the binocular part of the left visual field on the rostrolateral part of the right optic tectum and on the rostrolateral part of the left optic tectum, while the monocular part of the left visual field is represented only on the contralateral optic tectum.

The results show that the vertical and horizontal co-ordinates of the visual field undergo a geometrical transformation in the course of projection on to the tectum. It was possible to determine the number of microns of tectum devoted to one degree of visual field measured radially from the fixation point, the magnification factor (M) of Daniel & Whitteridge (1959). M does not decrease at the same rate along the vertical and horizontal meridians. Along the vertical meridian, M decreases from a maximum of 18·9 μ/degree, 20° below the fixation point, to a minimum of 7·7 μ/degree at the superior periphery of the vertical meridian. Along the horizontal meridian, M decreases from a maximum of 15·0 μ/degree 50° nasal to the fixation point, to a minimum of 10·7 μ/degree at the temporal periphery.

The region of the visual field which has the most magnified central representation forms a horizontal band stretching across the visual field just below the horizontal meridian. This corresponds with the position of the area centralis retinae in the frog described by Chievitz (1889).

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Comparison of nystagmoid responses to rotational stimuli about vertical and rolling axes. By G. M. JONES. *R.A.F. Institute of Aviation Medicine, Farnborough, Hants*

Using the method of suddenly stopping a prolonged turn of constant angular velocity (stopping stimulus), Henriksson (1955) has demonstrated correlation between the slow-phase angular velocity of nystagmus and the vestibular stimulus. Hallpike & Hood (1953) have shown how this angular velocity decreases exponentially with respect to time elapsed after the stopping stimulus. In both instances investigation was confined to stimuli about the vertical axis. The present experiment compares the slow-phase angular velocity of the nystagmoid response after stopping stimuli about two orthogonal axes, namely a vertical axis with head erect (horizontal nystagmus) and a rolling axis parallel to the visual axis (rolling nystagmus). Eight subjects were each exposed to a single stopping stimulus about each of these axes after turning for 3 min at 60°/sec. Electro-nystagmography, with eyes closed and with wide band-width d.c. amplifier, was employed for recording horizontal nystagmus and a previously described cine technique (Free & Jones, 1960) for rolling nystagmus. A separate experiment showed that, with this technique, optokinetic influence about the rolling axis was probably negligible.

The results confirm the exponential form of decay observed by Hallpike & Hood and establish a similar form of decay, but with a different time constant, about the rolling axis. The mean time constants for all subjects, obtained from calculated regression lines, were 16·4 sec (S.D. 4·0) and 3·9 sec (S.D. 0·8) for the vertical and rolling axes respectively; a difference perhaps related to the difference in likely duration of rotational movement about these two axes in everyday life. However, the corresponding mean values of slow phase angular velocity immediately after stopping were similar to one another, although well below the velocity of the stimulus, being 28·5°/sec (S.D. 3·8) and 23·8°/sec (S.D. 4·2) for the vertical and rolling axes respectively. The fact that these values are statistically only just distinguishable from one another suggests that if a similar stimulus were applied with eyes shut about an intermediate axis, the axis of resulting slow phase nystagmus would *initially* be roughly parallel to that of the stimulus, despite the considerably reduced angular velocity of response. Since the initial values upon which this observation is based are presumably virtually

uninfluenced by the cupula-restoring force, it seems likely that a similar situation might obtain during the short angular head movements of everyday experience. The greater rate of decay observed about the rolling axis than the vertical axis, suggests that with more prolonged rotation about an intermediate axis, the axis of eye response would tend to move progressively towards the vertical axis of the head employed in these experiments, a conclusion supported by the subjective sensation experienced in these circumstances.

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Cooling and warming the cerebral cortex of the rat. By O. C. J. LIPPOLD and J. W. T. REDFEARN. *Department of Physiology, University College London*

Changes in temperature modify the electrophysiological properties of excitable tissues; in particular, cooling appears to give rise to depolarization in fine nerve terminals (e.g. mammalian muscle spindle afferents; Lippold, Nicholls & Redfearn, 1960). The effect of changes of temperature upon the activity of cortical dendrites has been investigated in the rat lightly anaesthetized with urethane. A trephine hole 5 mm diam. was

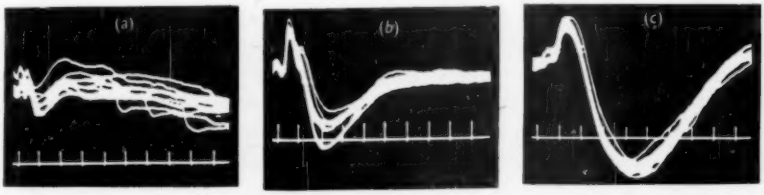

Fig. 1. Evoked potentials from rat cortex. Shock to cutaneous nerve at start of each trace, 10 consecutive traces superimposed. (*a*) Temperature of exposed, intact dura 2° C above body temperature; (*b*) 3° C below and (*c*) 11° C below it. Time marker 5 msec. Voltage 500 μV. Time constant 10 sec. Records (*a*), (*b*) and (*c*) were taken within 3 min.

made over the sensory cortex and records were taken, through the intact dura, of the evoked cortical responses to single shocks applied to a sensory nerve in the fore limb, at different temperatures of the brain surface.

Figure 1 shows the cortical evoked potential: (*a*) when the temperature of the exposed dura was about 2° C above, (*b*) 3° C below, and (*c*) 11° C below deep body temperature. When recorded in the cold, evoked responses had a longer duration and the magnitude of the negative wave was increased.

If the whole rat was cooled to 25° C there was little alteration in the size or shape of the evoked potential, although its latency was increased. From this it appears that the effects described are due to the thermal gradient across the brain surface rather than to the actual temperature of the cortex. Similar effects on the magnitude and sign of the potential were produced by passing a small direct current across the cortex; the second wave was often reversed in sign by surface-negative polarization of a few μA (cf. Bishop & O'Leary, 1950). The topical application to the cortex of Krebs's solution, at body temperature, containing 10–15 mM-K^+, also produced an effect on the amplitude and duration of the negative wave similar to that of cooling.

The size, shape and sign of the cortical response would appear to be sensitive to gradients in the membrane polarization of the superficial nervous tissue.

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Abolition and initiation of tremor by drugs injected into the cerebral ventricles of cats. By F. R. DOMER* and W. FELDBERG.
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In anaesthetized cats tubocurarine perfused from the lateral cerebral ventricle to the aqueduct produces tremor which is abolished by adrenaline or noradrenaline added to the perfusion fluid (Feldberg & Malcolm, 1959). We have now examined in cats the effect of intraventricular adrenaline, noradrenaline, and of other substances, on chlorpromazine and pentobarbitone sodium tremors. In addition, intraventricular 5-hydroxytryptamine (5-HT) was found to produce tremor.

Chlorpromazine tremor. Long-lasting tremor occurred 1–4½ hr after an intramuscular injection of chlorpromazine 25 mg/kg. It was abolished for 10–25 min by intraventricular injections of 5 μg adrenaline or noradrenaline. Larger doses caused longer-lasting cessation of tremor. Tremor was also abolished by intraventricular chloralose (200 μg) and $CaCl_2$ (300 μg), but not by intraventricular ephedrine, atropine or hyoscine (tested up to 500 μg).

Pentobarbitone sodium tremor. Shivering or tremor is known to occur in cats anaesthetized with pentobarbitone sodium (Hall & Goldstone, 1940; (Lippold, Redfearn & Vuco, 1959). According to Hall & Goldstone, this tremor is first accentuated but later abolished when adrenaline 80–150 μg/kg is injected intravenously. In our experiments tremor ceased for several minutes after an intraventricular injection of adrenaline, noradrenaline

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(5–10 μg), chloralose (200 μg) or $CaCl_2$ (350 μg), but not after ephedrine, atropine or hyoscine (tested up to 500 μg). Further, when perfusion was effected from the lateral ventricle to the aqueduct with artificial cerebrospinal fluid at a rate of 0·1 ml./min, tremor began to decrease after 1 min perfusion when the perfusing fluid contained adrenaline 1/1,000,000, or noradrenaline 1/250,000, or chloralose 1/50,000, or $CaCl_2$ 1/5000; i.e. when 0·1 μg adrenaline, 0·4 μg noradrenaline, 2 μg chloralose or 20 μg $CaCl_2$ had entered the ventricular cavities.

5-HT tremor. In conscious cats intraventricular injections of 5-HT 250–500 μg produced tremor lasting sometimes up to 2 hr.

In monkeys the regions from which tremor-like movements are elicited on electrical stimulation are the medial portions of the reticular formation in the mesencephalon and medulla (Jenkner & Ward, 1957). In cats the regions extend further forward into the hypothalamus (Birzis & Hemingway, 1957). The regions are those which contain high concentrations of noradrenaline (Vogt, 1954) and 5-HT (Amin, Crawford & Gaddum, 1954). These findings, together with the effects of intraventricular noradrenaline and 5-HT, suggest that one of the physiological functions of these substances in the brain-stem may be control of tremor activity.

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The action of some choline esters and other parasympathomimetic drugs on mammalian non-myelinated nerve fibres. By Christine J. Armett and J. M. Ritchie. *Albert Einstein College of Medicine, New York* 61, *N.Y., U.S.A.*

Acetylcholine depolarizes mammalian non-myelinated fibres, reduces their spike height, enhances the spike after-positivity and slows conduction (Armett & Ritchie, 1960). The present experiments were done to see how much this ability of acetylcholine to alter directly the electrical properties of nerve fibres was shared by other choline esters.

The whole of a rabbit's vagus nerve was desheathed and its action and resting potentials studied by using the sucrose-gap apparatus described by Stämpfli (1954). The nerve was perfused with Locke's solution during most

of the experiment; for short periods this Locke's solution was replaced by a similar solution containing acetylcholine or another test drug. The response to acetylcholine in a concentration of 3×10^{-4} g/ml. (i.e. 1·7 mM) was used as the standard with which to compare the responses to the other drugs. All drugs except pilocarpine nitrate and nicotine hydrogen tartrate were given as chlorides.

Carbachol (carbamoylcholine chloride) had much the same effect as acetylcholine; thus, in a typical experiment where acetylcholine 3×10^{-4} g/ml. reduced the spike by 35%, carbachol in the same molar concentration reduced it by 28%. However, other choline esters were relatively ineffective; thus larger concentrations (10^{-3} g/ml.) of methacholine (acetyl-β-methylcholine chloride) and bethanechol (β-methylcholine chloride urethane) had little or no effect. Choline itself had hardly any effect when compared with acetylcholine in equimolar concentrations; a higher concentration of 17 mM (i.e. 0·1 isotonic) did reduce spike height, but even at this high concentration the response was two or three times smaller than that produced by the standard concentration of acetylcholine (producing in a typical experiment a 12% fall in spike height compared with a 35% fall produced by acetylcholine).

These results with the different choline esters suggested that a nicotinic rather than a muscarinic effect of acetylcholine was involved. This suggestion was supported by the findings that the action of acetylcholine could be matched by a similar molar concentration of nicotine, but not even by larger molar concentrations of pilocarpine or of arecoline (an alkaloid which is qualitatively similar in its action to pilocarpine), these drugs in a concentration of 10^{-3} g/ml. producing about a third of the effect on spike height as that produced by acetylcholine 3×10^{-4} g/ml. Further support was given by the finding that the action of acetylcholine was much reduced or abolished by two drugs which are known to antagonize the nicotinic actions of acetylcholine at other sites, tubocurarine and hexamethonium (10^{-4}–10^{-3} g/ml.). However, it should be noted that the same concentration of atropine, a drug which blocks the muscarinic action of acetylcholine, is equally effective in blocking the action of acetylcholine on nerve fibres described here. The tubocurarine and hexamethonium did not by themselves alter the spike height; atropine (10^{-3} g/ml.) tended to reduce the spike height a little.

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Inhibition of the uptake of catecholamines in brain tissues by reserpine. By B. B. BRODIE, H. J. DENGLER, E. TITUS and C. W. M. WILSON*. *Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland, U.S.A.*

There is evidence that reserpine may block an active transport mechanism for the uptake of serotonin and catecholamines in the platelets (Hughes & Brodie, 1959), and it has been shown by Brodie, Titus & Wilson (1960) that there is no barrier to the uptake of various compounds by the pituitary and other areas of the brain from the blood. In view of the possible effects of reserpine on the transport of catecholamines in the brain, the uptake of adrenaline and noradrenaline by the pituitary and other parts of the brain has been compared with their uptake by muscle and liver *in vivo* and *in vitro*.

TABLE 1

	Tissue: plasma ratio			
	Adrenaline		Noradrenaline	
Cats	Normal	Reserpinized	Normal	Reserpinized
Cortex	1·7	0·4	4·4	1·3
Rhinencephalon	1·4	0·3	—	—
Hypothalamus	1·8	0·7	3·3	1·8
Thalamus	1·5	0·5	—	—
Mid-brain	1·4	0·6	—	—
Cerebellum	0·7	0·4	3·7	1·3
Pituitary	1·4	0·7	3·7	1·3
Pineal	4·4	2·2	—	—
Liver	0·8	0·2	1·5	1·4
Muscle	0·5	0·1	1·4	0·7
Plasma (μg/l.)	17		5	

Intravenous infusion of DL-epinephrine-7-H^3 for 30 min at a rate of 0·2–0·4 μg/kg/min resulted in plasma values of 2·7–3·8 g/l. and produced tissue: plasma ratios of 3·1–4·8 in the pituitary. Reserpine inhibited the concentrating mechanism of the pituitary so that ratios of 0·7–1·0 were obtained, which resembled those previously found in the pituitary for sulphaguanidine and *N*-acetyl 4-aminoantipyrine.

Slices of brain tissue, liver and skeletal muscle were incubated for 60 min in a mixture of Krebs's bicarbonate medium and serum (1:1) containing tritiated adrenaline or noradrenaline. The amines were isolated from the tissues by the method of Weil-Malherbe & Bone (1952). It was found that they were concentrated in normal brains, but that prior treatment of the cats with reserpine inhibited this mechanism as shown in Table 1. In a few experiments the pineal gland and area postrema also were shown to con-

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centrate adrenaline. The concentrating mechanism in the hypothalamus became saturated as the concentration of adranaline in the medium was increased.

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Site of the action of D-tubocurarine that enhances evoked cortical potentials. By A. B. CAIRNIE and J. L. MALCOLM. *Department of Physiology, University of Aberdeen*

Feldberg, Malcolm & Sherwood (1956) reported that an intraventricular injection of 200 μg D-tubocurarine produced a large increase in the negative phase of the electrical response evoked in the cortex and in the thalamus, in cats under chloralose anaesthesia, by peroneal nerve stimulation. The accompanying increase in muscular activity has been shown to be due to the effect of tubocurarine on the periaqueductal grey matter (Feldberg & Malcolm, 1959).

In the present experiments a solution of tubocurarine (1×10^{-3} mg/ml. of an artificial cerebrospinal fluid) was perfused at 4 ml./hr from the lateral ventricle to the cisterna and there was no change in the evoked cortical response. Nor was there an enhancement of the evoked cortical response following an intraventricular injection if the dura over the somato-sensory area were intact, although injection of dye suggested that the tubocurarine had reached most of the brain surface except the somato-sensory area. On the other hand, when a tubocurarine solution (1×10^{-3} mg/ml.) was perfused at 4 ml./hr from a needle inserted in the dura to the cisterna, the evoked response was increased only when the solution was shown to have reached the somato-sensory area.

The response evoked in the thalamus by the same stimulus was not enhanced when application of the tubocurarine was limited to perfusion of the ventricles, though the tubocurarine did produce some increase in the spontaneous activity.

The results indicate that the enhancement of the evoked responses does not depend upon excitation by the tubocurarine of structures close to the ventricles, but is, in the case of the cortex, dependent upon the movement of the c.s.f. containing tubocurarine to the somato-sensory area. Movement in this direction to a sufficient degree only occurs when the dura is opened over this region, when the rate of movement following an intraventricular injection is sufficiently rapid for an effect to take place in 3–4 min. On the other hand, if the dura is intact, a single intraventricular

injection or perfusion of the ventricles only occasionally produces a slight enhancement of the evoked cortical response after a delay of 30–40 min.

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The effect of barium on the release of acetylcholine from the cat's superior cervical ganglion. By W. W. DOUGLAS, D. W. LYWOOD and R. W. STRAUB. *Department of Pharmacology, Albert Einstein College of Medicine, New York* 61, *New York, U.S.A.*

The release of acetylcholine by impulses in cholinergic nerves is known to be influenced by calcium and magnesium. When calcium is removed from the Locke's solution used to perfuse the superior cervical ganglion, the output of acetylcholine on preganglionic stimulation falls (Harvey & MacIntosh, 1940). Conversely, the amount of acetylcholine liberated by stimulation increases with increasing calcium concentration, and the effect of calcium is antagonized by magnesium (Hutter & Kostial, 1954). Studies of end-plate potentials in skeletal muscle have indicated similar antagonistic actions of calcium and magnesium on acetylcholine release from stimulated motor neurones (see del Castillo & Katz, 1956).

We have now found that barium, another ion of the alkali earth group, has also pronounced effects on acetylcholine release. In our experiments cats were anaesthetized with chloralose and the superior cervical ganglion perfused with an oxygenated buffered Locke's solution which contained (mM) NaCl, 154; KCl, 5·6; $CaCl_2$, 2·2; Na_2HPO_4, 2·15; NaH_2PO_4, 0·85; Glucose, 10, or with similar solutions containing barium or free from calcium. The preganglionic trunk was stimulated at 2 shocks/sec for periods of 5 min alternating with 7 min periods of rest, and the acetylcholine content of the ganglion perfusates assayed on the blood pressure of the chloralosed, eviscerated cat. With the Locke's solution acetylcholine was found in successive stimulated samples in approximately equal amounts. Little or no acetylcholine was detected in the samples taken without nerve stimulation. When barium (6 mM) was added to the Locke's solution, the amount of acetylcholine released by nerve stimulation was approximately doubled without any apparent change in the resting efflux of acetylcholine. When the calcium in the perfusion fluid was replaced with an equimolar amount of barium, preganglionic stimulation continued to be effective in releasing acetylcholine for at least 40 min. After the acetylcholine output on stimulation had been reduced by perfusion with calcium-free Locke's solution for about 30 min, it was found that the addition of barium (2·2 mM) restored it to about the control values found with Locke's solution.

It has been suggested that the release of acetylcholine by nervous impulses is effected by the entry of calcium ions into the nerve fibre (Birks & MacIntosh, 1957; Hodgkin & Keynes, 1957). Barium perhaps acts in the same way as calcium: its action may thus provide a further clue on the acetylcholine ejection mechanism. It seems not unlikely that the stimulant effect of barium on acetylcholine output may underlie some of its many known pharmacological effects.

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Ether-soluble active lipid in rabbit brain extracts. By N. Ambache and Margaret Reynolds. *Medical Research Council, Department of Physiology, Royal College of Surgeons, London*

Extracts of bled or Locke-perfused cerebral hemispheres ground in distilled water of pH 7·8 (1 ml./200 mg tissue) and centrifuged, contracted hamster colon preparations (suspended in a 5 ml. bath in atropine 10^{-7} ± 2-brom-D-lysergic diethylamide bi-tartrate, BOL 148, 10^{-7}), in doses of ≡ 10–40 mg tissue, and were usually 5–8 times weaker than corresponding iris extracts.

The active material was purifiable through acetone. When crude extracts were partitioned with 1 vol. ether the activity recovered from the ether phase was < 10 % and 12·5 % at pH 8·54 and 8·14, but 66–90 % at pH 2·36–2·98 (6 experiments). However, when petroleum ether (B.P. 40–60° C) was substituted for ether < 5–10 % was recovered at pH 2·16–2·89.

Recovery was almost nil if the extracts were pre-treated with IBr before partition with ether at pH 2·36. Activity was also destroyed by phenyl isocynate. These findings suggest an unsaturated lipid with at least an acid and an OH (or amine) groups, and thus far resembling irin.

The rat uterus (in atropine and BOL 148) is 10 times less sensitive than the hamster colon but does respond to large doses both of ether-purified brain (≡ 400 mg) and of ether-purified irin (≡ 40–60 mg); it is insensitive to Na ricinoleate up to 188 μg/ml.

Extracts of other parts of the brain contained similar activity recoverable by acid partition with ether.

α-Hydroxy-nervonic and *N*-acetyl-neuraminic acids were inactive on the hamster colon.

Differential nerve block by sodium-deficient solutions. By P. W. NATHAN and T. A. SEARS. *The Medical Research Council Neurological Research Unit and the Institute of Neurology, National Hospital, Queen Square, London, W.C.* 1

The action of sodium-free and sodium-deficient solutions on conduction in fibres of the different groups has been studied on spinal roots of the cat. Peripheral nerves were stimulated and the compound action potentials recorded from filaments of the spinal roots (S 1–4). The cut end of the filament was threaded through holes in the sides of a chamber (internal diam. 8 mm, capacity 1 ml.) and then laid on recording electrodes. The chamber was filled with the different solutions, leakage being prevented by wax seals; liquid paraffin protected the roots from drying.

The sodium concentration of the 'normal' solution (a modified Krebs–Henseleit's solution after Diamond, Gray & Inman 1958), was 145 mM; sucrose, or in some experiments, choline chloride, was used to replace the sodium.

Sodium-free solutions caused complete block of conduction in all myelinated and non-myelinated fibres. This blocking occurs at different rates. Thus, a large proportion of the alpha beta fibres is still conducting when the gamma, delta, and non-myelinated fibres are completely blocked. Recovery of fibres of all sizes occurs within seconds of applying the normal solution and is complete within 1 min.

Solutions containing between 10 % and 25 % of the normal amount of sodium block the fibre groups differentially. For example, a solution containing 15 % of the normal amount of sodium blocks the gamma, delta, and non-myelinated fibres without blocking a significant proportion of the alpha beta fibres. Solutions containing 20 % of the normal amount of sodium block delta and non-myelinated fibres, but a proportion of the gamma fibres continue to conduct at diminished velocity. Slowing of conduction velocity is conspicuous with concentrations of sodium near to threshold for blocking, especially with non-myelinated nerve fibres. The non-myelinated fibres continue to conduct at diminished velocity, in a solution which blocks the very smallest myelinated fibres (25 % of normal sodium concentration). This result possibly depends on differences between saltatory conduction and conduction in a continuous system, rather than on fibre size.

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The effects of oestrogen, progesterone and placental extracts on inulin and para-aminohippurate clearances in the anaesthetized rat. By B. F. MATTHEWS. *Department of Physiology, University of Aberdeen*

We have shown (Matthews & Taylor, 1960) that both inulin and para-aminohippurate (PAH) clearances are increased in 14-day and 18-day pregnant rats anaesthetized with barbiturate when compared with unmated control rats, and have suggested that the placentas were involved in the increased clearances found during pregnancy. The present experiments were designed to discover more direct evidence for this hypothesis.

The methods used for measuring the clearances in the anaesthetized rat were as described previously (Matthews & Taylor, 1960). The effect of oestrogen and progesterone was investigated by measuring the inulin and PAH clearances in three groups, each consisting of eleven ovariectomized rats. The first group was injected subcutaneously with oestradiol benzoate (10 μg daily) dissolved in olive oil; the second group received the same amount of oestradiol plus progesterone (2 mg daily) in olive oil, and the third group olive oil alone. Injections were made twice daily and on the 14th day the clearances were measured. Values for inulin and PAH clearances were found to be similar in the groups receiving oestradiol or olive oil alone, but in the group given both oestradiol and progesterone the clearances were slightly lower.

The effect of oestrogen and progesterone was also investigated in three further groups, each of nine rats. Pregnancy was terminated on the 12th day and the three groups were then injected as in the first experiment. Inulin and PAH clearances were measured after the injections had been given for 6 days. No significant differences in the clearances of the three groups was found, and it is concluded that in the present experiments oestrogen alone or combined with progesterone has little effect on inulin and PAH clearances.

A more direct approach to the problem was made by investigating the effect of placental extracts on inulin and PAH clearances. In two groups, each of eight rats, pregnancy was terminated on the 12th day. One group was injected twice daily for 6 days with a suspension in saline of homogenized whole placentas from 14-day pregnant rats; the other group was similarly injected with the supernatant fluid of the centrifuged saline extract of homogenized placentas from 14-day pregnant rats. Each rat was given the equivalent of one to two placentas daily. A third group of six received saline alone. Clearance values were apparently slightly higher in the groups injected with suspension of placentas or supernatant fluid compared with those injected with saline alone. For PAH the differences

were not significant. For inulin the differences were just outside the 5 % level of significance ($P = 0{\cdot}05$–$0{\cdot}1$). The results though inconclusive suggest that there may be something in the placenta affecting the inulin clearance values.

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The percentage of noradrenaline in the adrenal glands of families of cats. By K. R. BUTTERWORTH and MONICA MANN. *Department of Pharmacology, St Mary's Hospital Medical School, London, W. 2, and School of Pharmacy, London, W.C. 1*

A range of 13–91 % of noradrenaline in the adrenal glands of a group of thirty-eight cats has been obtained by Butterworth & Mann (1957), thus confirming the fact that the proportion of noradrenaline to adrenaline in cat adrenal glands varies considerably. In an attempt to investigate this variability, Butterworth & Mann (1960) have studied the adrenal catechol amine content of litters of healthy, young cats. All the cats in each litter were killed on the same day, the adrenal glands removed and extracted, and the extracts assayed biologically. It was found that, although there was a wide range in the percentage of noradrenaline (8–84 %) between the litters, there was little or no variation within a litter. This was found to be true in all the seventeen litters studied, and was independent of the size of the litter, the sex and the general appearance of the animals.

In a continuation of this work, each of six litters was subdivided. Intervals of 3–16 weeks were allowed between the removal of the glands from the cats in each subdivision. All animals were kept under as similar conditions as possible and no cat was used until it had been in the animal house for at least 3 weeks. It was found that, despite the interval of several weeks between the adrenalectomies, the percentage of noradrenaline in the glands of each litter remained constant.

With eight litters it has been possible to determine the percentage of noradrenaline in the glands of the mother cat. It was found that there was always good agreement between the values obtained for the mother and for her litter.

In four cases the percentage of noradrenaline in successive litters from the same mother was determined. Two groups containing two litters each and two containing three each, were obtained. As might be expected from the mother-litter relationship, the values for the successive litters from each mother were similar.

The application of these results is that it is possible, in experiments on

the adrenal medulla of cats, to perform individual experiments on littermate cats at varying time intervals, each litter being subdivided into test and control groups. This obviates the necessity to compare the catechol amine contents of the left and right gland of each animal, one gland being removed as the control before the experiment is commenced.

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The effect of pituitrin on sweating. By W. S. S. LADELL and H. W. WHITCHER. *Chemical Defence Experimental Establishment, Porton Down, Wilts*

When inherently acclimatized Africans working at 1·7 kcal/m²/min in 31·5° C dry bulb (D.B.), 28° C wet bulb (W.B.) were given a pharmacological dose of pituitrin those sweating heavily sweated less and those sweating lightly sweated more (Ladell, 1948). An attempt has been made to confirm this on unacclimatized Europeans; twenty-four unacclimatized Europeans were exercised at 1·7 kcal/m²/min for 2 hr on 4 successive days in 32° C D.B., 26·5° C W.B., air movement *c.* 15 m/min. On 2 of the 4 days, selected at random, each man was given 5 units of pituitrin i.m. immediately on entering the climatic chamber. All the men were given 1000 ml. of water to drink 10 min before exposure. The mean urine outputs during exposure were: control tests, 414 ml., thirty-seven observations only, diuresis was inhibited by heat (Weiner, 1944) on eleven occasions; pituitrin tests, 70 ml., including nil flows on twenty occasions. There was no statistical difference between the mean 2 hr sweat productions either in quantity or in range: control 1030 g ± S.D. 298 g, test 1092 g ± S.D. 299 g. The sweat gland responses (ratios of the sweat produced to the over-all rises in rectal temperature, 'r.t.') were, however, markedly diminished in the first hour after pituitrin: mean control sweat gland responses were 699, 387 and 315 g/° C above initial r.t. for the three 20 min periods 10–30, 30–50 and 50–70 min respectively; mean post-pituitrin responses, 511, 288 and 261 g/° C also respectively. These lower responses after pituitrin were associated with higher r.t.'s Thus at 50 min the mean r.t.'s were: control, 37·5° C ± S.D. 0·33° C; post-pituitrin, 37·8° C ± S.D. 0·29° C ($t = 4·9$, $P < 0·001$).

These observations suggest that any effect pituitrin in pharmacological doses may have on sweating is indirect. The peripheral vasoconstriction produced by pituitrin (Aitken & Priestley, 1925) will tend to reduce cutaneous heat losses, hence r.t.'s will rise more quickly during work in

heat. With higher r.t.'s the sweat responses are reduced, consequently in severe conditions when the r.t. rises above about 38° C (Ladell, 1957) the total sweat production will be diminished. No evidence can be adduced from this experiment of any direct physiological effect of endogenous ADH on the sweat glands.

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Bone strength and calcium retention of rats in hypervitaminosis A. By A. Khogali*. *Physiology Department, University of St Andrews at Queen's College, Dundee*

The occurrence of spontaneous fractures in rats fed with excessive amounts of vitamin A was first reported by Collazo & Rodriguez (1933). and since then has been confirmed by many workers. The common site of these fractures is the upper third of the tibia. The cause of such fractures, however, is still obscure.

The following table summarizes the results of an experiment in which young rats were given vitamin A acetate in graded doses for 4 weeks. Only the rats receiving 24,000 i.u. of vitamin A per day developed spontaneous fractures. The bones from this group showed reduced breaking stress (see Bell, Cuthbertson & Orr, 1941) without any alteration in the ash content.

		Vitamin A (i.u./day)			
	Control	24,000 i.u.	12,000 i.u.	6000
No. of rats ...	6	6	6	6
Body weight (g)				
Initial	55	54	53	54
Final	157	89	157	167
Femur weight (g)	0·323	0·159	0·297	0·332
$\frac{1}{4}Wl$ (Lb.in.)	3·02	0·96	2·24	2·79
$\frac{1}{4}Wl$ (kg. cm)	3·48	1·11	2·53	3·21
Breaking stress (Lb./sq.in. $\times 10^3$)	33·5	24·1	33·5	33·3
Breaking stress (kg/cm^2 $\times 10^3$)	2·36	1·71	2·36	2·32
Ash in tibia %	53·6	53·7	54·7	55·0
Total calcium retention (g)	1·07	0·38	0·97	1·10

Microradiographs of undecalcified sections of bone showed no difference in mineralization between hypervitaminotic and normal rats. If l is the length of bone between end supports, and W the weight applied to the centre, the bending moment is $\frac{1}{4}Wl$ (Bell *et al.* 1941): the bending

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moment at breaking was reduced in all the groups receiving vitamin A, the reduction being particularly marked in the group which sustained spontaneous fractures during the experiment.

The total calcium retention was significantly lowered in the severely hypervitaminotic group in which the bones were reduced in thickness. Histologically, these bones showed trabeculated endosteal bone. Whether the thin bones seen in hypervitaminosis A are due to acceleration of the remodelling process or whether the vitamin interferes in some way with the availability of calcium to the animal remains to be investigated.

Vitamin A acetate was kindly supplied by Messrs Glaxo Laboratories Ltd.

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Quantitative effects of β-aminopropionitrile on bone growth.

By G. H. BELL and D. N. SHARMA.* *Department of Physiology, University of St Andrews at Queen's College, Dundee*

Recently weaned rats were fed on rat cake and given β-aminopropionitrile fumarate (APN) by gavage. After 4 weeks they were killed and the physical properties of the femora were measured (Bell, Cuthbertson & Orr, 1941). The breaking stress fell from about 33,000 Lb./sq.in. (2320 kg/cm^2) in the controls to about 5000 Lb./sq.in. (344 kg/cm^2) in those receiving 108 mg APN daily. The fall in breaking stress with high dosage of APN cannot be attributed to an alteration in the quality of the bone material laid down, since the cross-sections of the bones show increasing trabeculation with increasing dosage. In other words, it seems that the fall in breaking stress is a measure of the sponginess of the bone and not necessarily of deterioration in the material. Nevertheless, it seems justifiable to use the data to draw a dose–response curve and to use it to find in quantitative terms the effect of certain alleged antagonists to APN, such as thyroxine.

We are indebted to Abbott Laboratories Ltd. for a supply of APN and to Glaxo Laboratories Ltd. for a supply of thyroxine.

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Metabolism of progesterone by homogenates of the liver of new-born mice. By K. BROWN-GRANT and E. FORCHIELLI. *Department of Physiology, University of Birmingham and the Worcester Foundation, Shrewsbury, Mass., U.S.A.*

Karnofsky, Hamre & Hysom (1952) have reported that progesterone is toxic to new-born but not to adult mice. A failure of 'detoxification' by the liver was postulated. Metabolism of progesterone by reduction of the double bond in ring A involves a loss in biological activity and the enzymes responsible are found in the liver. Their activity may be measured *in vitro* by anaerobic incubation of homogenates at pH 7·2 in the presence of a reduced Co II generating system, followed by extraction of the added steroid and the determination of the absorption at 240 mμ. The level of

TABLE 1. Δ^4-Hydrogenase activity of mouse-liver homogenates. Values are means ± standard error of mean. Fifteen experiments were performed with homogenates of sucklings' livers and seven experiments with homogenates of adults' livers

	Percentage reduction in 20 min			
	Sucklings		Adults	
Steroid	Per 50 mg wet wt.	Per 2 mg N_2	Per 50 mg wet wt.	Per 2 mg N_2
Progesterone	38±3	47±4	32±3	38±4
Cortisone	5±1	6±1	11±2	12±1
Testosterone	13±1	16±2	18±4	19±4

activity is much lower in homogenates of the livers of young rats than of adults (Forchielli, Brown-Grant & Dorfman, 1958). The livers of mice of the Webster strain from litters less than 24 hr, 2, 3, 4, 5, 6 and 7 days of age were pooled and homogenized. Steroid Δ^4-hydrogenase activity was determined in triplicate by the methods of Forchielli *et al.* using progesterone, cortisone or testosterone as substrate. Two or more determinations on tissue from litters of each age and on tissue from adult animals of the same strain were carried out. Results from male and female adults did not differ significantly and were combined, nor did the results from litters of different ages which were also combined. Table 1 shows that no differences in the rate of reduction of progesterone between homogenates from suckling and adult mice were detected. Both preparations reduced progesterone more rapidly than either cortisone or testosterone. There is thus no evidence that toxicity of progesterone in new-born mice is due to deficiency of liver enzymes carrying out ring A reduction. In the mouse the liver content of steroid Δ^4-hydrogenases appears to be as high in new-born as in adult animals. The reason for this difference from the rat is not known.

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The staircase effect shown by the myometrium. By BRENDA M. SCHOFIELD. *Physiology Department, Royal Veterinary College, London, N.W. 1*

Csapo & Corner (1952) demonstrated that the staircase effect occurs in uterine muscle. In isolated strips of rabbit uterus, the staircase effect is influenced by ovarian hormones, an oestrogen-dominated myometrium having a positive staircase whereas a progesterone-dominated myometrium has a negative staircase. Schofield (1954) showed that the staircase effect could also be elicited in the intact rabbit, the results of experiments *in vivo* being equivalent to those *in vitro*.

At the end of pregnancy in the rabbit, changes in hormonal dominance can be demonstrated by recording the staircase effect (Bengtsson, 1957; Schofield, 1957). Thus a negative staircase effect, indicating progesterone domination, is demonstrable during pregnancy until 1 or 2 days before term. Towards the end of pregnancy the progesterone influence declines and some 24 hr before parturition the myometrium, becoming dominated by oestrogen, shows a positive staircase effect.

A similar phenomenon occurs in the pregnant ewe (Bengtsson & Schofield, 1960). These findings in the intact ewe were confirmed on isolated uterine strips. Thus the hormone domination at the end of pregnancy appears to be similar in the sheep and rabbit.

In the intact pregnant rat, on the other hand, recordings over the last 8 days of pregnancy, during parturition and immediately post partum showed a negative staircase effect; a positive staircase effect was never elicited. This was confirmed on isolated strips.

In all three species the myometrium became more sensitive to oxytocin with the approach of parturition. In the rat and rabbit, the tension developed in response to electrical stimulation increased towards the end of pregnancy: in the rabbit during the last 2 weeks and in the rat during the last few days.

The staircase effect was investigated in the ovariectomized rat injected daily with ovarian hormones (up to 100 μg of oestrogen for 4–12 days, which is several times the dose necessary in the rabbit to demonstrate a positive staircase effect (Schofield, 1955)). A positive staircase effect was never obtained and the staircase effect was usually negative. This was not altered by simultaneous progesterone injection.

It is evident, therefore, that even with high levels of oestrogen and in the absence of progesterone, the myometrium of the rat does not, under these experimental conditions, show a positive staircase effect. This suggests that at the time of parturition in the rat there is not necessarily a pattern of hormonal control different from that found in the sheep and rabbit. It seems more probable that this discrepancy is due to a different organization of the myometrium, so that the responses of the rat myometrium to electrical stimulation are not the same as in the other two species.

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Stimulation of heat production in new-born cats and adult rats by adrenaline and noradrenaline. By H. BAUM, R. E. MOORE and MARY C UNDERWOOD. *Royal Free Hospital School of Medicine, London W.C.* 1

The calorigenic properties of noradrenaline (NAd) in the new-born small mammal have been previously reported (Moore & Underwood, 1959, 1960*a*, *b*). Adrenaline (Ad) is inactive in this respect in the new-born. A calorigenic response to Ad does, however, develop with age, but even in the adult is small compared to the effect of NAd on the new born. These observations, together with an observed decline in the calorigenic potency of NAd with age, posed a problem of considerable interest.

It appeared to us that a likely explanation could be sought in the ease of penetration to calorigenic effector sites. The following experiments were designed to enable us to test the hypothesis that both NAd and Ad were calorigenic provided that they could reach the site of metabolic stimulation in both the new-born and adult mammal.

New-born cats and adult rats were given subcutaneous (s.c.) injections of dibenzyline (25 mg/kg), followed after $\frac{1}{2}$–1 hr by s.c. Ad or NAd (400 μg/kg). Oxygen consumptions and, in the kittens, body temperatures, were measured as previously described. At an environmental temperature of 35° C, dibenzyline had no effect on oxygen consumption (or on kitten body temperature), but the subsequent injections of either NAd or Ad were calorigenic in both new-born cat and adult rat. Ad and NAd were found to be of approximately equal potency under these conditions.

We consider that the action of dibenzyline in potentiating these effects

was due to its blocking of the surface receptors for Ad and NAd. This prevented the rapid removal from the blood of the injected catecholamines and thus permitted their access to the calorigenic effector sites. Once there, metabolic stimulation became apparent.

We therefore suggest that the calorigenic effect of injected NAd declines with age because of the development of surface receptors which can effectively remove NAd from the blood. The implications are further that Ad receptors are well developed in the neonate and that some of these might change their specificity towards NAd as the animal matures.

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Uterine distension and the vaginal cycle in the guinea-pig.

By B. T. DONOVAN and W. TRACZYK*. *Department of Neuroendocrinology, Institute of Psychiatry, Denmark Hill, London, S.E.* 5

Distension of the uterus of the sheep by the insertion of plastic beads is associated with changes in the length of the oestrous cycle, which are not evident if the segment containing the bead is denervated (Nalbandov, Moore & Norton, 1955). Nervous stimuli arising from the uterus would thus appear to be of significance in the regulation of the oestrous cycle of the ewe. Little comparable work has been carried out in other species and therefore the effect of distension of the uterus on the vaginal cycle of the guinea-pig has been studied.

Under barbiturate anaesthesia two glass beads (7 mm long and 3 mm diameter) were inserted through the cervical canal into each horn of the uterus at various stages of the oestrous cycle. They were pushed as far toward the Fallopian tubes as possible. Control operations, in which beads were inserted and removed, were also performed. The oestrous rhythm was followed by daily examination of the vaginal membrane and a smear was taken if the vagina was open.

It was found that the immediate effect produced by the insertion of a bead varied according to the stage of the cycle at operation. The most consistent feature of the results is that the introduction of beads into the uterus 10 days or more after the appearance of a cornified smear often delayed the next oestrous period. This effect was not evident when the beads were inserted about the time of oestrus. If the beads remained in the uterus during subsequent cycles then these were generally of shorter duration than normal.

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These observations seem to indicate that the timing of oestrus in the guinea-pig may be altered by manipulation of the uterus.

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The selective appetite for Na^+ in sheep. By D. A. DENTON and J. R. SABINE. *Department of Physiology, University of Melbourne, Victoria, Australia*

Some sheep, when offered solutions of Na^+ salts to drink, ingested voluntarily considerable amounts though they were in normal Na^+ balance. The establishment of a permanent unilateral parotid fistula caused loss of 1–4 l. of alkaline saliva/day containing 180–700 m-equiv of Na^+—mainly $NaHCO_3$. This Na^+ loss caused a large increase of appetite for Na^+. The voluntary intake of many animals was adequate to maintain a normal Na^+ balance, whilst others oscillated around a balance involving some degree of residual Na^+ deficiency. If solutions of both NaCl and $NaHCO_3$ were offered (e.g. 420 m-equiv/l.) the animal showed a clear preference for $NaHCO_3$, a more appropriate choice in the face of loss of alkaline parotid saliva. If the fistulated sheep were offered continuous access to $NaHCO_3$ solution and water, and the concentration of the $NaCHO_3$ solution was varied from 119 to 940 m-equiv/l., the animal varied the volume drunk so that intake remained relatively constant and approximated the loss from the fistula. If the Na^+ solutions were available to the sheep for only 15–60 min each day, the animal usually ingested enough to correct its deficit. Eighty to ninety per cent of intake occurred during the first 5 min. If access were permitted only on every 2nd or 3rd day, the amount of Na^+ ingested was increased commensurately. The frequency and procedure of sampling the solutions during normal Na^+ balance and Na^+ deficiency was compared during short-term access. There was a large increase of sampling activity during Na^+ deficiency. The sheep rarely ingested any KCl solution when it was included amongst the solutions offered. The preference for $NaHCO_3$ over NaCl was maintained during this regime of short-term access. Na^+ deficiency produced changes of behaviour. Distance receptor information indicating impending presentation of Na^+ solutions caused increased salivary secretion and other evidences of medullary excitation.

The further purification of gastrin: secretory responses in a human subject. By R. A. GREGORY and HILDA J. TRACY. *The Physiological Laboratory, University of Liverpool*

Gregory & Tracy (1959) reported the preparation from hog antral mucosa of gastrin in a form free from histamine, which stimulates gastric acid secretion when injected subcutaneously, as well as intramuscularly or intravenously, into conscious dogs provided with gastric fistulae or fundic pouches. The product described, though potent and non-toxic, proved unsuitable for attempts at further refinement by chromatographic or electrophoretic techniques; it consists, like the preparations of earlier investigators (Munch-Petersen, Rönnow & Uvnäs, 1944; Uvnäs, 1945; Jorpes, Jalling & Mutt, 1952) largely of denatured protein, which is insoluble at pH 4–8 and adsorbs gastrin when precipitated. The problem of removing this protein has now been solved by precipitation of it with piperidine and acetic acid from solution in 80% acetone. A fourfold increase in potency results without loss of gastrin and the product can be fractionated on a column of calcium phosphate gel (Gregory, 1955). This retains the gastrin completely; almost all of the inert biuret-reacting material in the solution applied can be removed by washing. The gastrin is then completely displaced by a low concentration of phosphate, preferably used in the form of a linear gradient; the activity emerges as a sharp peak corresponding to a concentration of 0·01–0·02% PO_4 entering the column. This fraction contains a trace of pigment and the 'tail' of it is inactive. Paper chromatography and electrophoresis of the active 'front' portion shows that several components are present. An amount of this fraction corresponding to 20–40 g mucosa (a suitable dose for a dog) contains 0·01–0·02 mg total N; it is closely similar in activity and properties to the cruder material previously described (Gregory & Tracy, 1959).

Amounts of this purified material corresponding to 200 g mucosa (0·1 mg N) and derived from the same or different preparations were injected subcutaneously, intramuscularly or intravenously into the same human subject on different occasions. Secretion commenced within 15 min and after subcutaneous or intramuscular injection lasted for over 3 hr; a typical output was 465 ml. containing 44 m-equiv total HCl. After intravenous injection the response was much smaller and lasted only about 2 hr. No definite evidence of increased pepsin output was obtained. The subject experienced no significant side- or after-effects.

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Feeding responses in separated fundic pouches in dogs before and after isolation of the innervated antrum. By MAUNG PE THEIN and BRENDA M. SCHOFIELD. *Department of Physiology, Medical School, King's College, Newcastle upon Tyne* 1

The ability of the vagus nerve to release gastrin must now be accepted, but the contribution of this process to the normal acid response to feeding is still uncertain. If secretion be collected by the irrigation method described by Burstall & Schofield (1953), Heidenhain gastric pouches usually show a clear response to feeding beginning in the first 15 min and attaining a peak within the first hour. This is contrary to the experience of many workers using conventional techniques for collection, but is similar to that of Gregory & Tracy (1960) who used totally denervated Heidenhain pouches. There is thus a place for a 'nervous phase' in the response of the Heidenhain pouch to feeding.

It is of course uncertain how much and how soon the direct effects of food in the stomach contribute to the early response, but some information can be obtained by isolating the antrum as a vagally innervated pouch. In both ordinary and totally denervated Heidenhain-pouch preparations this procedure, which should largely eliminate the direct effects, usually enhances the early phase of the feeding response. Oberhelman, Rigler & Dragstedt (1957) also observed that Heidenhain pouches showed a marked acid response within 15–30 min after feeding following isolation of the innervated antrum. Acidification of the antral pouch strongly inhibits the early feeding response. Vagal denervation of the antral pouch greatly reduces or abolishes it.

These results suggest that a large part of the early feeding response of the Heidenhain pouch may depend on vagal release of gastrin and may not require contact of the food with the antral mucosa. The very small acid secretion of ordinary Heidenhain-pouch preparations to sham feeding is of no significance in assessing the contribution of this process to the normal feeding response, since the antrum is exposed to unbuffered acid secreted by the body of the stomach. This inhibitory influence seems unlikely to operate strongly after normal feeding with the antrum *in situ*. Woodward (1960) has recently stated that the critical pH for antral inhibition appears

to be about 1·7, while according to Thomas (1940) the antral pH in dogs following a meal of meat normally lies between 2·0 and 3·0 with a node at 2·6.

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Observations on the blood carbon-dioxide dissociation curves of emphysematous patients. By R. W. B. Penman and O. L. Wade.
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PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY

LIVERPOOL MEETING

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A device for the continuous measurement and recording of intravascular pH. By E. S. JONES, D. V. ROBERTS and J. S. ROBINSON. *Departments of Medicine, Physiology and Anaesthesia, University of Liverpool*

Technique of urine collection in the hen. By A. H. SYKES. *Department of Physiology, University of Liverpool*

Simultaneous recording of the flow of digesta through the duodenum with movements of the stomach in ruminants. By A. G. SINGLETON. *The Physiological Laboratory, University of Liverpool*

Flow through the duodenum is recorded and measured by a modification of the electromagnetic method previously used for measuring blood flow in unopened vessels (Katz & Kolin, 1938; Richards & Williams, 1953). Animals are prepared with a re-entrant duodenal fistula, so that duodenal contents can be passed through a tube fixed in the field of a large permanent magnet. The digesta, acting as a conductor, induce a potential difference across the tube which is proportional to velocity, and is positive or negative according to the direction of the flow. As the tube is rigid and of uniform cross-sectional area, velocity is proportional to the volume flowing. The voltage change is recorded photographically from an oscilloscope, and volume of flow is calculated from measurements of the areas between velocity trace and base line, after direct calibration.

The tracings confirm Phillipson's observation (1952) that flow occurs mainly in gushes, but show that reverse flow also occurs, so that a proportion of the material is returned to the stomach.

Stomach movements are recorded by means of a balloon and water manometer, the latter being modified to act as a variable condenser. The water manometer is encircled on the outside by a sheet of metal foil, and is filled with a weak salt solution. The glass acts as the dielectric, and movement of the fluid varies the capacity. The change in capacity is recorded

by conventional methods on the oscilloscope. As a recording method it has no advantages over balloons and water manometers, but it enables a tracing to be recorded electrically which is comparable to those obtained by using smoked paper.

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A machine for shaking tubes vertically. By S. A. HEMES and P. T. NOWELL. *Department of Pharmacology and General Therapeutics, University of Liverpool*

The relation between age and response to drugs of the isolated phrenic-nerve diaphragm of the young rat. By J. B. ROBERTS. *Department of Pharmacology and General Therapeutics, University of Liverpool*

A non-corrodable valve for use in automatic control of flow of physiological fluids. By S. A. HEMES and J. B. ROBERTS. *Department of Pharmacology and General Therapeutics, University of Liverpool*

A method for estimating neostigmine (prostigmin) and pyridostigmine in urine. By P. T. NOWELL, CAROL A. SCOTT and A. WILSON. *Department of Pharmacology and General Therapeutics, University of Liverpool*

Neurological and hormonal mechanisms involved in experimental gastric ulceration. By K. MARTINDALE, G. F. SOMERS and C. W. M. WILSON. *Department of Pharmacology and General Therapeutics, University of Liverpool, and The Distillers Company (Biochemicals) Limited, Fleming Road, Speke, Liverpool* 24

Both nervous and hormonal factors influence the production of gastric ulcers. In rats the central-nervous-system depressants, phenobarbitone, meprobamate and chlorpromazine, are ineffective in preventing restraint ulcers. The sedative drug thalidomide has some form of specific action against restraint ulcers and in inhibiting a stress component induced by segregation in phenylbutazone ulcers. We have found that vagotomy induces ulceration in rats, which has also been reported in dogs (Keller, 1936) and this is inhibited by adrenalectomy. While haemorrhagic

restraint ulcers are potentiated by adrenalectomy, ulceration is not prevented by hydrocortisone but the haemorrhagic component changes.

These results show that the ulcers produced by adrenal and by vagal mechanisms have different characteristics.

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Micro-incineration technique applied to cytological investigations. By J. KRUSZYNSKI. *Department of Histology, University of Liverpool*

Tissue-section artifacts due to incomplete removal of embedding media. By J. KRUSZYNSKI. *Department of Histology, University of Liverpool*

Electron microscope studies of *Trichinella spiralis* infecting mouse striated muscle. By EVELYN B. BECKETT and B. BOOTHROYD. *Department of Histology, University of Liverpool*

Electron microscope investigations on the osteoclast. By B. BOOTHROYD and N. M. HANCOX. *Department of Histology, University of Liverpool*

Effect of parathyroid hormone on avian bone in tissue culture. By N. M. HANCOX and SYLVIA S. WARNER. *Department of Histology, University of Liverpool*

Smooth-muscle activity in the lung of the frog. By D. V. ROBERTS. *Department of Physiology, University of Liverpool*

Pinocytosis in osteoclasts *in vitro* (film). By N. M. HANCOX. *Department of Histology, University of Liverpool*

The effect of bradykinin in an isolated perfused dog lung preparation. By B. A. WAALER. *University Laboratory of Physiology, Oxford*

Bradykinin is known to be a powerful vasodilator substance in the systemic circulation of various species, including dog and man (Rocha e Silva, Beraldo & Rosenfeld, 1949; Fox, Goldsmith, Kidd & Lewis, 1960). Collier, Holgate, Schachter & Shorley (1960) showed that the substance

produced marked bronchoconstriction both in isolated perfused guinea-pig lungs and on intravenous injection in the urethane-anaesthetized guinea-pig. There is, however, little further information available on the effect of bradykinin in the lungs.

The effect of bradykinin was tested in an isolated, ventilated dog lung-lobe preparation, perfused by a method slightly modified from that previously described by Daly (1956). Autologous blood was used as perfusate and the tidal air was measured. Pure bradykinin (Elliott, Lewis & Horton, 1960), or a partly purified substance was used.

Bradykinin (3–10 μg) injected into the pulmonary artery cannula produced a 5–20% fall in pulmonary vascular resistance (PVR) in seven experiments. Larger doses did not produce a greater fall in PVR. The minimal effective dose ranged from 0·5 to 5 μg of pure bradykinin. A gradual reduction of the responses occurred on repeated injections. Atropine did not affect the pulmonary vasomotor response to bradykinin.

In some preparations (4 out of 7) bradykinin caused a slight bronchodilatation, increasing the tidal air by less than 5%. Bronchoconstrictor responses never occurred.

The addition to the perfusate of a small amount of dog's saliva, which is known to contain a bradykinin-forming enzyme, caused a fall in PVR of the same magnitude and duration as did an injection of a large dose of bradykinin. Addition of a small amount of human saliva, however, caused severe bronchoconstriction and vasoconstriction in the preparation.

Partly purified and pure bradykinin samples were kindly supplied by Dr G. P. Lewis.

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The cytochrome c content of the heart and skeletal muscle of iron-deficient rats. By H. A. Salmon. *Department of Physiology, University of Sheffield*

As reports of the effect of iron deficiency upon the cytochrome c content of animal organs are conflicting a further study was undertaken. Male albino rats, 45–50 g in weight, were fed on an iron-deficient diet; controls were given also ferrous sulphate. They were kept in newly galvanized cages and analysed when the iron-deficient animals became suitably anaemic. Haemoglobin was estimated as cyanmethaemoglobin and cytochrome c by the method of Rosenthal & Drabkin (1943). Recovery of

added cytochrome c was greater than 90% and spectrophotometry revealed no absorption bands other than those of cytochrome c.

The mean body weight (4 pairs of animals) of the iron-deficient group (mean Hb = 3·4 g/100 ml.) was 142 g and that of the controls (mean Hb = 13·4 g/100 ml.) was 172 g, but this difference was not significant, $P > 0{\cdot}05$. The mean heart weight (0·781 g) of the iron-deficient animals was significantly greater than that of the controls (0·525 g), $P < 0{\cdot}01$, despite the disparity in body weight. Histological examination with Oil Red O and Scarlet Red revealed no excess of fat in the hearts of the iron-deficient animals.

The mean total cardiac cytochrome c of the iron-deficient animals (288 μg) was significantly greater than that of the controls (192 μg), $P < 0{\cdot}05$, and the mean concentration was not significantly different in the hearts of the iron deficient (366 μg/g) and control (360 μg/g) group, $P > 0{\cdot}1$. In skeletal muscle the mean cytochrome c concentration in the iron-deficient animals (30 μg/g) was significantly less than that of the controls (75 μg/g), $P < 0{\cdot}01$, but the mean water content of this tissue was not increased significantly (75·7 and 73·8% respectively), $P > 0{\cdot}1$. The mean kidney and liver cytochrome c concentrations were not significantly reduced in the iron-deficient group.

As the total cardiac cytochrome c increased proportionately with the heart weight in these anaemic animals, it is provisionally suggested that this represents a useful intracellular adaptation to iron deficiency. The organ presumably performing more mechanical work than usual (by analogy with cases of anaemia in man) is maintained in one of the substances necessary for the production of the energy required; the fact that skeletal muscle was not so affected supports this interpretation. The results are consistent with the view that cytochrome c may be rate-limiting under some circumstances (although this subject is controversial) and may be regarded as an 'adaptive' enzyme.

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Inhibition of some hepatic mechanisms by chronic biliary obstruction. By W. H. H. ANDREWS and I. DEL RIO LOZANO. *Department of Pharmacology, St Mary's Hospital Medical School, London, W.2*

Ligature of bile ducts was carried out in rats either 40 min (acute obstruction) or 2–3 weeks (chronic obstruction) before an intravenous injection of sulphobromophthalein (BSP), 20 mg/kg weight. Experiments were performed under anaesthesia with ether and pentobarbitone. Blood was withdrawn by cardiac puncture 40 min after dye injection, and the liver

was then removed. The dye concentrations of liver and plasma were estimated colorimetrically and acetone extracts were subjected to paper chromatography.

The plasma of all rats contained both unchanged and metabolized, probably conjugated, BSP, the proportion of metabolized being slightly greater in acute than in chronic obstruction. The concentration of dye in plasma was greater in acute obstruction. In the liver, the BSP present was nearly all metabolized and the amount present in acute obstruction was twice that present in chronic obstruction, although chronic ligation of the bile ducts produced a doubling of the liver weight. There therefore appeared to be failure of a mechanism which causes concentration of BSP by the liver. This was confirmed *in vitro*.

Livers were perfused at 37° C with BSP in Ringer–Locke solution, and the clearance of dye was much less in chronic than in acute obstruction. It was also noted that the transudate escaping from the surface of the liver was of the same dye concentration as the hepatic venous outflow in chronic obstruction, whereas in acute obstruction the transudate was considerably more concentrated than the outflow. No difference was noted in the uptake of BSP by slices of liver from normal rats and from rats with obstructed bile ducts or by control and experimental livers perfused at room temperature. In another series of experiments (to be published) it has been shown that both slicing and cooling appear to inhibit a dye-concentrating mechanism normally present in the liver.

In some rats the obstruction of the bile duct was released, and there was an immediate flow of bile. After acute obstruction, bilirubin and BSP were present in the bile but after chronic obstruction they were absent. Chronic obstruction of the bile duct therefore appears to inhibit a dye secretion and a dye concentrating mechanism (which may be related) but does not inhibit fluid excretion or greatly alter BSP metabolism.

The work was supported by a grant from the Medical Research Council.

The effect of back-pressure upon bile flow rate. By T. G. Richards and J. Y. Thomson. *The Physiological Laboratory, University of Liverpool*

It is well known that, in the absence of the gall-bladder, if the common bile-duct is cannulated, bile will be secreted up a vertical tube. In the dog the flow of bile ceases at a height of about 30 cm above the hilum of the liver. It is also stated that the flow rate is unvarying until the height of the column is very near to the maximum, i.e. the 'secretory pressure'.

The statements above are found to hold only if the diameter of the tube employed is large, i.e. greater than 8·0 mm, with a control bile flow

rate of approximately 0·15 ml./min in the anaesthetized dog. If the diameter of the tube is approximately 2 mm or 3 mm the rate at which bile rises in the tube is not constant. In such a case there is initially a rapid and almost linear decrease in rate, which is followed by a steady ascent at a reduced rate, and this rate is maintained until the column has risen almost to its maximum.

Experiments have been made in order to relate the pattern of change described above to (*a*) tubes of different diameter in the same animal, and (*b*) to varying degrees of induced choleresis in the same tube.

The findings lead to the conclusion that the pressure–volume characteristics of the biliary tree play an insignificant part in the appearances. It seems that the rate of ascent of bile, and its relation to the rate of increase of some force which brings about the continued excretion of bile, is all-important. Where the tube is wide and ascent is slow, the necessary increase in 'excretory force' to raise the column one centimetre higher is attained in the time available; where time is insufficient for this, ascent of bile in the column decreases until its rate (in the tube employed) matches the rate of increase in excretory effort.

If some progressive increase in secretory effort takes place in these conditions, a further result may be expected in the case of bile flowing along a horizontal tube placed in the plane of the hilum. In these conditions a pressure applied which is constant in value and acts against flow will lead first to a reduction of flow, then to a predictable rate of increase, and finally to a restoration of the control flow rate. This has been confirmed at all applied pressures unless they were at or near to the 'secretory pressure'.

A simple model has been constructed which fulfils the requirements of the statement above, and which will give a good approximation to the *in vivo* results.

Bile secretion in the conscious sheep. By F. A. Harrison* and K. J. Hill. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

By exteriorizing the bile flow from the gall-bladder to the arm of duodenum, the method described by Hill & Taylor (1957) and Taylor (1960) for the collection of pancreatic juice was modified to allow the collection of bile from the conscious sheep. During experiments in which consecutive hourly collections were made for 24–30 hr, bile from the gall-bladder was allowed to drain freely into a container fastened to the animal's side.

Experiments on Clun Forest sheep, which received a daily ration of 1000 g hay chaff and 200 g oats, showed that the volume and total solids (TS) of the bile fell during the first 3 hr of collection and then continued

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at an average rate of 0·4 ml./kg/hr (TS 2·90 g/100 ml.). Emptying the reticulo-rumen reduced the secretory rate to 0·25 ml./kg/hr (TS 1·80 g/100 ml.).

The return of bile to the animal by continuous slow infusion into the duodenum modified the secretory pattern. Thus when bile (TS 3·05 g/100 ml.) was infused at 30 ml./hr the decline in volume and total solids of the bile secreted during the first 3 hr was less than observed previously and thereafter secretion continued at a higher level (0·62 ml./kg/hr, TS 3·75 g/100 ml.). This effect was dependent on the amount and TS of the bile returned and was probably attributable to its bile-salt content since a similar response was obtained by the infusion of a bile-salt preparation (Oxo Ltd., London). The marked drop in TS observed when bile was drained from the animal was therefore probably related to the removal of bile salts from the circulation.

Although these experiments provided an indication of the secretory activity of the liver, TS of gall-bladder bile obtained at post-mortem was always greater than that of bile collected from the gall-bladder cannula, and it appeared that in this preparation there was partial interference with the concentrating capacity of the gall-bladder. The volume of bile which normally enters the duodenum is therefore likely to be less than that obtained during the present experiments.

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Sweat gland failure on the body surface and on the forearm enclosed in an arm bag. By K. J. COLLINS and J. S. WEINER. *Medical Research Council Climate and Working Efficiency Unit, Department of Human Anatomy, University of Oxford*

Eccrine sweat glands exhibit a relatively high resistance to failure during continuous stimulation by low concentrations of acetylcholine (ACh), although high concentrations rapidly desensitize the glands (Collins, Sargent & Weiner, 1959). After 6 hr of thermal sweating the response to methacholine is reported to decrease (Thaysen & Schwartz, 1955) and this has been taken as evidence for 'fatigue' of the sweat glands. In the present experiments on seven subjects in environments of 46° C dry bulb/30° C wet bulb or 40° C dry bulb/32° C wet bulb, it was found that the sweat response to ACh did not decrease significantly, that sweat rate decreased by less than 30% of the initial maximum, and that skin and rectal temperatures increased steadily during 3–5 hr of heat exposure. The decline in sweating is enhanced by increasing the humidity of the environ-

ment when, at high rates of secretion, sweat drips unevaporated from the skin surface (Gerking & Robinson, 1946).

A much more rapid failure of forearm sweating was, however, induced by enclosing the limb in an impermeable envelope. After 2–3 hr, sweating in the arm bag diminished by 70–100% (mean 92%) of the maximum output, while on the body surface the decrement was 11–27% (mean 19%) after 3–4 hr. The response to ACh in the arm bag was also considerably reduced. Sweat gland activity recovered gradually when the arm bag was removed during heat exposure and a normal reaction to ACh could usually be elicited 1 hr after removing the arm bag in a cool environment. It can be shown that the higher skin temperature in the arm bag alone is not responsible for the pronounced depression in sweating. In the conditions of high humidity and high skin temperature which prevail in the arm bag, part of the sweat adhering to the skin is probably reabsorbed. The slight swelling of the forearm and the high osmotic pressure of sweat collected in arm bags (Weiner & van Heyningen, 1951) is evidence of this. One explanation for the failure of sweating in the arm bag may be that excessive hydration obstructs the sweat ducts. If this is so it would explain the more rapid onset of 'fatigue' in humid than in dry conditions.

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Two-stage transfer of glucose by the intestine. By J. Matthews and D. H. Smyth. *Department of Physiology, University of Sheffield*

Newey, Parsons & Smyth (1959) have shown that the main action of phlorrhizin on intestinal transport of glucose is to prevent entry of glucose into the cell from the mucosal side, and they have pointed out that this entry mechanism may include the active transfer mechanism, or alternatively there may be a separate mechanism for transfer distinct from the entry mechanism. Experiments have been undertaken to investigate these possibilities. The experimental device was the sac of everted small intestine of the rat (Wilson & Wiseman, 1954). The sacs were filled initially with 1 ml. bicarbonate saline containing 500 mg glucose/100 ml., and suspended in 15 ml. of the same solution, and incubated for 1 hr aerobically, anaerobically or in the presence of phlorrhizin or dinitrophenol.

The results of experiments with the middle part of the intestine were as follows, the terms used being those of Parsons, Smyth & Taylor (1959). Phlorrhizin (10^{-3} M) abolished serosal transfer. It also nearly completely

abolished mucosal glucose transfer and gut glucose uptake. Anaerobic conditions and 2:4-dinitrophenol (2×10^{-4} M) both completely abolished serosal glucose transfer, but did not prevent entry of glucose into the cell from the mucosal side. Although this was reduced there was still a large gut glucose uptake, which is mostly due to metabolism. Hence the action of phlorrhizin differed from that of anoxia or dinitrophenol, in that it not only inhibited movement against a concentration gradient, but also prevented entry of glucose into the cell from the mucosal side.

It is thus evident that since anoxia or a high concentration of dinitrophenol do not prevent entry of glucose into the cell, the entry mechanism is not dependent on aerobically produced metabolic energy. The entry mechanism is, however, sensitive to phlorrhizin, and hence there must be a phlorrhizin-sensitive part of the mechanism involved in glucose transfer which is different from the part which is dependent on aerobic metabolic energy. The results do not prove that abolition of aerobic energy production may not have some effect on entry of glucose, nor that phlorrhizin has not also an effect on the energy-requiring mechanism, but they show that there must be two distinct mechanisms.

Since the phlorrhizin-sensitive mechanism does not depend on metabolic energy, it could well be a facilitated diffusion, and possibly be concerned only with entry of glucose into the cell. For active transport against a concentration gradient a second stage is involved which requires metabolic energy, and it is this stage which is chiefly affected by anoxia and 2:4-dinitrophenol.

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A description of cardiac pace-maker potentials based on the Hodgkin–Huxley equations. By D. NOBLE*. *Department of Physiology, University College London*

At a previous meeting of the Society it was shown (Noble, 1960) that the quantitative formulation of the ionic hypothesis given by Hodgkin & Huxley (1952) to describe the action potential in squid nerve requires only small modifications to make it applicable to the much longer-lasting action potentials of cardiac muscle. The equations used to describe the sodium current closely resembled Hodgkin & Huxley's, those for h (the variable describing the availability of sodium carriers) being based on Weidmann's (1955) experiments on Purkinje fibres. The potassium current equations were based on Hutter & Noble's (unpublished) measurements

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of the voltage–current relations of Purkinje fibres in Na-deficient solutions. They found that the potassium conductance *falls* when the membrane is depolarized, but that part of this fall is transient. To describe this behaviour, two sets of equations were used. One was a simple empirical equation describing an instantaneous fall in conductance on depolarizing the membrane. The other was a modification of Hodgkin & Huxley's equations for delayed rectification in which both the magnitude of the conductance increase and the speed of onset were made very small. In these equations there is one potential at which the steady-state sodium and potassium currents are equal and opposite. If its value is high enough it forms the resting potential and the 'membrane' is stable unless excited.

It will now be shown that by inserting appropriate values for the constants in the equations for m (the variable describing the activation of sodium carriers) the system may be made unstable in diastole and pace-maker activity then occurs. The behaviour of this computed pace-maker has been tested by observing the effect of increasing the 'potassium' conductance and also by adding various degrees of 'anion' conductance, the anion equilibrium potential being set below the maximum diastolic potential (Hutter & Noble, 1959; Lamb, 1960) at -60 mV.

A small increase in 'potassium' conductance is sufficient to arrest activity completely, which is in accordance with the extreme sensitivity of the natural pace-maker to the increase in potassium conductance produced by acetylcholine (Hutter & Trautwein, 1956). The effect produced by adding an 'anion' conductance depends greatly on its magnitude: 0·075 m-mho produces a 50 % increase in frequency but has only a small effect on the maximum diastolic potential and, although the action potential is shortened, its general form is unaltered. 0·2 m-mho greatly increases the frequency of the pace-maker, reduces the maximum diastolic potential and abolishes the overshoot of the action potential. This closely resembles the long-term effect of nitrate ions on the ectopic pace-maker in Purkinje fibres (Hutter & Noble, 1959). By contrast, 0·4 m-mho stops pace-maker activity completely, which may be compared to the arrest of spontaneously beating Purkinje fibres produced by iodide ions (Hutter & Noble, 1959).

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The influence of barbiturate on thyroxine binding by serum proteins. By W. E. Balfour and H. E. Tunnicliffe. *Physiological Laboratory, University of Cambridge*

Using paper electrophoresis and a barbiturate buffer, Gordon, Gross, O'Connor & Pitt-Rivers (1952), found that in human serum thyroxine is bound to a post-albumin protein fraction (T.B.P.) whose rate of migration is between those of the α_1 and α_2 globulins. Ingbar (1958) in similar experiments but with tris-maleate buffer and Balfour & Tunnicliffe (1959) with borate-phosphate buffer and zone electrophoresis on cellulose columns found that three thyroxine-binding zones are clearly differentiated. In addition to T.B.P. and albumin a fast-moving pre-albumin zone was shown to bind thyroxine.

Now, a comparison has been made between the thyroxine distributions seen after zone electrophoresis on cellulose columns when either borate-phosphate or barbiturate buffers at pH 8·6 were used. With each buffer we find that pre-albumin can be easily separated and it is present in human serum at a concentration of $\sim$ 25 mg/100 ml. In the presence of barbiturate buffer a very small, but measurable, amount of thyroxine is bound to the pre-albumin. On the other hand, in borate-phosphate a large fraction of the thyroxine is pre-albumin-bound. When this pre-albumin-bound thyroxine is dialysed against barbiturate buffer the thyroxine is released from the protein.

Tata & Shellabarger (1959) have shown that when duck serum is subjected to paper electrophoresis in barbiturate buffer, added thyroxine is bound by the albumin and a post-albumin (T.B.P.) is not evident. We have confirmed this observation by zone electrophoresis on a cellulose column with barbiturate buffer. With borate-phosphate buffer, however, a large fraction of the thyroxine migrates with the pre-albumin protein zone. Since the amount of pre-albumin separated was the same with each buffer, we conclude that barbiturate interferes with thyroxine binding by pre-albumin.

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Leptomeningeal collateral circulation in *Macacus rhesus*. By L. SYMON. *The National Institute for Medical Research, Mill Hill, London, N.W.* 7

In macaque monkeys anaesthetized with sodium thiopentone, leptomeningeal collateral circulation was studied by injecting ^{32}P-labelled red cells into the right carotid circulation, and observing their passage through an area of right parietal cortex with a probe counter before and after occlusion of the right middle and anterior cerebral arteries.

After the middle cerebral artery was occluded there was at first little evidence of flow. During this initial period, however, division of a small pial artery resulted in a free though slowed flow of blood. This is in agreement with findings of Petersen & Evans (1937) and Meyer (1958). When observations were repeated at intervals during the next $\frac{1}{2}$ hr, collateral flow was seen, in a number of animals, to develop gradually via the ipsilateral anterior cerebral artery. Such collateral flow developed promptly after middle cerebral occlusion if both vertebral arteries had been ligated 1–2 hr previously.

The poor collateral flow immediately after middle cerebral artery occlusion was not seen in corresponding experiments on dogs (Symon, 1959). Anatomical differences in the course of the anterior cerebral arteries of these two species may account for this. Partly, however, it seems to result from transient spasm which is known to occur on manipulation of the basal vessels (Harvey & Rasmussen, 1951). In the present experiments, the reduction in calibre of these vessels when pronounced, could be seen by naked eye. When not so pronounced, it could be detected by reduction in trace height and slight delay in the arrival of labelled red cells injected shortly after the release of a temporary occlusion of the middle cerebral artery. The spasm disappeared in from 25 to 40 min.

After the middle and anterior cerebral arteries were occluded no flow could be detected in the observed cortical area from carotid injection. In a few experiments, however, in which the labelled red cells were injected directly into the right vertebral artery, clear evidence of flow was obtained, indicating that residual flow after middle and anterior cerebral occlusion arose at least in part from the vertebro-basilar circulation.

Perfusion from the aorta with carmine gelatine confirmed these findings. After clipping the middle cerebral artery, there was little reduction in the intensity of perfusion of the ipsilateral cortex, or basal ganglia. When its perforating branches, however, were occluded in addition by diathermy, the greater part of the head and body of the caudate nucleus and the putamen of the lentiform nucleus failed to be perfused. On the other hand, the globus pallidus and tail of the caudate nucleus remained well perfused, which is in contrast to the findings in dogs (Symon, 1959) and is explained

by the fact that in macaques the anterior choroidal artery, supplying these areas, springs from the internal carotid artery.

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Potassium and hydrogen-ion content of histamine-stimulated gastric secretion in the cat. By E. L. BLAIR, A. A. HARPER, D. HARRIS, J. D. REED and R. WILKINSON. *Department of Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne* 1

It has been claimed that there is no correlation between the potassium and hydrogen-ion concentrations of histamine-stimulated gastric secretion in man (Werther, Parker & Hollander, 1960) and in dogs (Hollander & Colcher, 1960). From these observations it has been concluded that the secretion of hydrochloric acid and of potassium ions are independent processes. The present results were obtained on cats, anaesthetized with chloralose and with the vagus and splanchnic nerves cut.

The stomach was intubated via the oesophagus and the pylorus occluded. Fifty ml. of a glycine solution (0·3 osmolar and pH 6·2) were run into the stomach and histamine acid phosphate given by slow intravenous injection. The glycine solution was replaced at intervals and the outputs of hydrogen and potassium ions were estimated by electrometric titration to pH 6·2 and by flame photometry respectively. In a number of experiments, to eliminate contamination by oesophageal or pyloric secretions, the glycine solution was confined to the acid-secreting area of the stomach by ligatures at the cardia and at the junction of the body and pyloric antrum. This modification did not affect the pattern of the results.

A highly significant positive correlation was found between the total outputs of potassium and hydrogen ions in secretory responses lasting 1–2 hr ($r = 0{\cdot}935$, $P < 0{\cdot}001$). As the evidence for the lack of correlation of the concentrations of potassium and hydrogen ions in histamine-stimulated gastric secretion in dog and man is greater at the beginning and end of the histamine response (Colcher & Hollander, 1959; Riddell, Strong & Cameron, 1960), the outputs of these ions in successive 10 min or 15 min periods have been analysed. Significant positive correlations were observed between potassium and hydrogen-ion output when the secretory response was well established and when it had fallen to low levels. At the beginning of the response the ratio of potassium to hydrogen was greater, and in the

period after the cessation of histamine injection it was less, than during the remainder of the response.

The correlation between the outputs of potassium and hydrogen ions when the secretory response is well established supports the view that there is a constant ratio of potassium and hydrogen ions in the primary acid secretion in response to histamine stimulation. It is possible to advance a hypothesis to explain the increased ratio of potassium output to hydrogen-ion output which occurs at the beginning of the response and the decreased ratio which occurs after the cessation of the histamine injection.

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Acetylcholine in regenerating motor nerves. By J. DIAMOND and C. A. N. EVANS. *Department of Physiology, University College London*

Lumbosacral ventral roots of anaesthetized cats were crushed intradurally under aseptic conditions. Two to twenty days later the regenerating roots (length 3–4½ cm) and normal ones of the opposite side were re-exposed and transferred to oxygenated buffered physiological saline. With a multi-blade knife the roots were cut into 5 mm portions whose acetylcholine (ACh) content was assayed, after a conventional trichloroacetic acid extraction, on the blood pressure of an anaesthetized eviscerated cat. The ACh content per 5 mm length (120–1150 pmoles, equivalent to about 24 μg ACh chloride/g wet tissue) was approximately constant over the normal and the proximal regenerated portion of the roots; ACh was not detected at the distal end of roots in experiments with short regeneration intervals (8–13 days). The transition between normal ACh content and very low or non-detectable content usually occurred over 5 mm length, at a distance from the crush which approximated to that predicted for maximum regeneration.

The effects of K^+-rich solutions (50–100 mM K^+) on ACh release from ventral roots have been investigated: such solutions had little or no effect on the relatively large spontaneous diffusion of ACh from normal roots; they did cause a release of ACh from regenerating fibres, whose resting output of ACh was very low.

Corticofugal discharges evoked by surface-anodal and surface-cathodal stimulation of the baboon's brain. By J. E. C. HERN,* S. LANDGREN,† and C. G. PHILLIPS. *University Laboratory of Physiology, Oxford*

In lightly anaesthetized baboons the pia-covered Rolandic cortex was stimulated with measured rectangular currents, using a ball-pointed focal electrode and a distant earth electrode, both non-polarizable. Synaptic potentials, recorded intracellularly from ulnar, median and radial motoneurones of C7 and C8 segments, were the index of corticofugal discharge. Stimulation was always well below threshold for firing the motoneurones unless these were already depolarized by injury. The actions of surface anodal (S+) and surface cathodal (S−) stimuli were compared.

With S+ shocks of 5·0 msec duration the threshold for minimal synaptic action ranged from 0·1 to 0·7 mA for different motoneurones. Depolarization reached its peak about 8·0–10·0 msec from the beginning of the shock. With stronger shocks this peak was larger. Depolarization started about 2·5 msec from the beginning of the shock. For motoneurones whose cortical S+ threshold was low, the S− threshold was always higher and the S− latency longer. When weak S+ shocks were repeated at 50 c/s, the synaptic potentials followed them without increasing in size and no synaptic action outlasted the stimulation period. With repetitive S− the synaptic potentials usually did grow, and an irregular synaptic activity often continued after the shocks had ceased. These results show that S+ is a more direct excitant of pyramidal neurones, and that S− probably excites them by an intracortical system showing temporal summation and after-discharge.

Brief (0·2 msec) S+ pulses had to be stronger than 5·0 msec pulses to produce equivalent depolarization. The latency was still about 2·5 msec, but the rising phase was now similar to that of the monosynaptic potential evoked by stimulation of Group Ia afferents to the same motoneurone. These potentials followed repetitive cortical stimulation at 420 c/s. The earliest positivity in the pyramidal tract at C8 level, which presumably preceded the arrival of the earliest pyramidal impulses, was 1·5–1·7 msec. Thus, although the arrival of those impulses which actually generated these synaptic potentials cannot be timed, it is concluded that this excitatory pathway is monosynaptic (Bernhard, Bohm & Petersen, 1953).

Inhibitory polarization has often followed the crest of the initial excitatory depolarization in response to single brief anodal shocks. Its latency was 3·5–4·0 msec from the start of the shock. Inhibition was best

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seen, however, when four or five such shocks were given at 200 c/s. The successive troughs of inhibitory polarization were then larger. The proportion of excitatory and inhibitory action was different when different cortical loci were stimulated. From some cortical sites an almost pure inhibitory polarization was evoked.

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Sensitivity to motion on a parallel swing of deaf children, of paraplegic patients and of normal subjects submerged in water. By E. G. WALSH. *Department of Physiology, University of Edinburgh*

Repetitive activity of muscle in the forelimb of the frog. By D. V. ROBERTS. *The Physiological Laboratory, University of Liverpool*

The flexor carpi radialis muscle in the male frog is larger than the corresponding muscle in the female, the difference in size probably being related to its participation in the clasp reflex. In addition to the size difference between the muscles in the two sexes, there appears to be in the male a specialization of function in the muscle fibres of the flexor carpi radialis. Contractions of the flexor carpi radialis muscles from both male and female frogs, and of the gastrocnemius muscle from male frogs, in response to indirect electrical stimulation have been recorded with an RCA transducer valve and cathode-ray oscilloscope, and certain differences in function between the male flexor carpi radialis and the other muscles named have been observed. The duration of contraction in the male flexor carpi radialis was longer than in the other muscles tested, and this difference was increased after brief motor nerve tetanization, when the shape of the myogram suggested the existence of two phases of activity, a fast response lasting approximately $\frac{1}{3}$ sec and a slow response lasting approximately $1\frac{1}{3}$ sec. The slow response was found to be facilitated by repetitive stimulation at 1–2 sec intervals and when tested at less frequent intervals, the facilitatory effect of previous tetanization declined over a period of approximately 10 sec. After neuromuscular block with curare, direct electrical stimulation of small groups of muscle fibres (one to five in number) in the male flexor carpi radialis produced the slow type of response after each stimulus. Electromyographic recording from the surface of this muscle showed that the prolonged contraction was associated with repetitive action potentials in the muscle fibres in response to single electrical

stimuli applied indirectly to the motor nerve, or directly to the muscle fibres themselves after the neuromuscular junctions had been blocked with curare. Intracellular recordings from single muscle fibres with glass capillary micro-electrodes confirmed the occurrence of repetitive firing after direct electrical stimulation, and showed that the action potentials arose from a maintained depolarization of the muscle membrane of about 30 mV.

It appears from these results that there exists in the membrane of the male flexor carpi radialis muscle fibres a specialized mechanism acting to amplify the effect of nerve activity on the contractile mechanism of the muscle fibres.

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